

EVOLUTIONARY TRAITS IN LIFE CYCLES
AND GILL DEVELOPMENT IN ODONATA

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- III. Norling, U., 1981. Photoperiodic responses and larval development in Leucorrhinia dubia (Van Der Linden) (Anisoptera: Libellulidae). A comparison between larvae from northern and southern Sweden. - Manuscript.
- IV. Norling, U., 1981. The life cycle and seasonal regulation of Coenagrion hastulatum (Charp.) in two climatically different areas (Odonata: Zygoptera). - Manuscript.
- V. Norling, U., 1981. Structure and ontogeny of the lateral abdominal gills and the caudal gills in Euphaeidae (Odonata: Zygoptera) larvae. - Zool. Jb., Anat. in press.

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- I. Norling, B., 1971. The life history and seasonal regulation of Parasitoid wasps in Sweden (Hymenoptera). Entomol. scand. 2: 119-130.
- II. Norling, B., 1976. Seasonal regulation in Leucospidius (Hymenoptera: Ichneumonidae) (Anisotoma: Ichneumonidae). - Entomol. scand. 2: 119-130.
- III. Norling, B., 1981. Phenological responses and larval development in Leucospidius (Hymenoptera: Ichneumonidae) (Anisotoma: Ichneumonidae) in relation to the life history of the host. A comparison between larvae from northern and southern Sweden. Entomol. scand. 2: 119-130.
- IV. Norling, B., 1981. The life cycle and seasonal regulation of Leucospidius (Hymenoptera: Ichneumonidae) in two climatically different areas (Hymenoptera: Ichneumonidae). - Entomol. scand. 2: 119-130.
- V. Norling, B., 1981. Structure and ecology of the lateral abdominal gills and the dorsal gills in Leucospidius (Hymenoptera: Ichneumonidae). - Entomol. scand. 2: 119-130.

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EVOLUTIONARY TRAITS IN LIFE CYCLES AND GILL DEVELOPMENT IN ODONATA

Most Odonata species occur in tropical or warm temperate areas. Thus, recent species well reflect the tropical origin of the order (e.g. DANKS, 1978). The life-cycles and their mechanisms of regulation in tropical and subtropical species are rather poorly known. There is, however, a considerable knowledge about these matters in many temperate-zone species (CORBET, 1980), and even some of the few species that penetrate to the highest latitudes have now been investigated in northern areas (III-IV; NORLING, 1975; KHARITONOV, 1977). From these studies it is now possible to discuss life-cycle patterns in temperate zone Odonata from evolutionary aspects.

In general, Odonata species require comparatively high temperatures for development in all stages (e.g. CORBET et al., 1960; DEACON, 1979) and long periods of cold and bad weather may be one of the worst enemies of the adult population (CORBET, 1962). It appears that also non-diapausing eggs may be most sensitive to low temperatures, at least in some phases of their development (DEACON, 1979). Even a constant temperature as high as $+15^{\circ}\text{C}$ can be lethal in temperate-zone species (RIVARD et al., 1975). In actively developing larvae, the late part of each larval stadium, where the moulting cycle takes place may be the principal cold-sensitive periods (III).

Thus, adaptation to a temperate climate requires that emergence is restricted to a part of the season when reproduction, and any associated cold sensitive stages, can be completed under favourable conditions, and that the winter is encountered in a resistant stage. The position of the resistant, often diapausing stage (stages) in the life-cycles varies, and an attempt towards a classification of odonate life-cycles in northern areas according to this has been made by NORLING (1975). The present studies (I-IV) allow a more detailed analysis of some of the life-cycle patterns involved in the colonization of higher latitudes, and some general conclusions also receive support from the data presented.

A fixed univoltine life-cycle with an obligatory, well defined diapause stage for overwintering seems to be one of the principal pathways of adaptations to a temperate climate. It simultaneously solves most problems of seasonal regulation (CORBET et al., 1960), but an additional estivation diapause may contribute in warmer areas,

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reminding of tropical-subtropical species (CORBET, 1980; UEDA, 1978). The hibernation stage is usually the egg stage, as in many Lestes species and Sympetrum species (CORBET, 1980), and in Aeshna mixta LATR. (MÜNCHBERG, 1931), but in a few species it may be the immature adult, as in Sympetrum species (CORBET, 1962). These cold hardy stages seem to have preadaptive parallels in drought-resistant stages of tropical forms (CORBET, 1962), and the evolution of this life-cycle pattern can be easily envisaged (CORBET et al., 1960). The rapid larval growth of these forms, often associated with a colonization of temporary waters, may allow a successful colonization of areas with relatively short summers, despite their obligatory univoltinism.

Most Odonata colonizing the temperate zone have, however, primarily evolved their life-cycle along another line, where the larval stage has become the resistant overwintering stage. In some instances, there may be evolutionary connections with the previously mentioned type of life-cycle (cf. CORBET et al., 1960), e.g. in forms where an egg diapause is also present (cf. the life-cycle variations described by SCHALLER, 1962).

The possibility to overwinter in a wide spectrum of larval instars gives a great adaptability to different climatic conditions, and allows a great variation in adult phenology. Special mechanisms for seasonal regulation of the appearance of the adult are necessary and usually take the form of an environmentally regulated restriction of development in later instars during the late part of the season (see below). Two phenological extremes, principally related to different winter population structures, can be discerned in accordance with the well-known classification of CORBET (e.g. 1962). These are (A) the "spring species pattern", where the winter before emergence is predominantly, or exclusively, spent in the final larval instar, and the emergence is early and highly synchronized (e.g. II-III), and (B) the "summer species pattern", where the last winter is spent in several instars without dominance of the final one. Emergence is later in the year and less well synchronized (e.g. I). There is, however, no sharp distinction between these two types (e.g. IV; NORLING, 1975; PAULSON & JENNER, 1971).

These patterns of seasonal regulation are determined by responses to photoperiod and temperature, and their interaction. Photoperiod affects development by induction and termination of diapause

(cf. IV, p. 6) or diapause-like phenomena of different intensities. There are three basic effects of photoperiod on development in Odonata, all well developed in the northern species examined here.

The first one is a prolongation of development by long-day photoperiods (long-day diapause). This most crucial response in seasonal regulation has been reported from many species (I-IV; CORBET, 1980). It is the first and most important step to prevent a too late emergence. The stages where the long-day diapause occurs, and the diapause intensity can vary greatly between species and, to some extent, between populations (I-IV). The prolongation of development is usually more conspicuous in one or two of the later instars. It is absent or not so readily apparent in early instars (II, IV, unpublished observations on Aeshna juncea (L.)). In early emerging species ("spring species") as Leucorrhinia dubia (VAN DER LIND.) (II-III) the long-day diapause may not be readily apparent until in the final instar. In many species and populations the long-day diapause produces an accumulation of larvae in the penultimate instar towards the end of the summer. These larvae enter the final instar towards the autumn, when moulting activity is stimulated temporarily by short, or particularly, intermediate daylengths (e.g. IV; LUTZ, 1974b). South Swedish populations of late flying species ("summer species"), as Aeshna viridis EVERSM. (I) and cyanea (MÜLL.) (unpublished data), have a relatively conspicuous summer diapause in several late instars, which largely prevents that the winter will be spent in the final instar.

The second basic photoperiodic response is a prolongation of development by short-day photoperiods (short-day diapause). It is the last step in the prevention of a too late emergence, and it simultaneously serves the survival of winter (hibernation diapause). The intensity of this diapause varies between species and instars. There may be a continuous transition from quiescence (a direct effect of unfavourable conditions on development) in smaller larvae to an increasingly intense and early induced diapause in late instars, which probably is related to seasonal regulation (synchronization of emergence; SCHALLER, 1962; ELLER, 1963; cf. I). However, there may also be a connection with an increase in the duration of the cold-sensitive moulting cycle in the later instars (cf. III).

As the long-day diapause, the short-day diapause have probably originated early during the colonization of temperate latitudes as

part of the mechanism for seasonal regulation. A regulation of the adult, reproductive stage, and probably also the egg stage, may have been necessary already at a stage of colonization when the winters still could be survived by larvae in a state of quiescence. When winters became longer and colder, some anticipatory responses, preventing that the winter is encountered during the sensitive moulting cycle, probably evolved. If the decrease in temperature during the autumn was slow, and the moulting cycle of short duration, a higher temperature requirement for the initiation of the moulting cycle than for its completion -- a most primitive temperature induced diapause -- would serve this purpose. Possibly, the winter quiescence of younger instars of many species is of this kind.

However, when the moulting cycle is of long duration, and when the temperature falls rapidly in the autumn, photoperiod is a better source of temporal information for a sufficiently early induction of diapause in each instar (cf. III). During colonization of higher latitudes, the diapause response to short days, primarily evolved for seasonal regulation, probably was extended to earlier instars as a pure hibernation diapause (cf. I-IV). In northern populations of Aeshna juncea (L.), all overwintering instars (approximately the 5-15th instars) show a typical short-day diapause, (unpublished data, but cf. NORLING, 1975).

The evolution of the specific hibernation diapause, which is well developed in the studied species, have probably also been connected with an increase in resistance to oxygen lack and subzero temperatures (SAWCHYN & GILLOTT, 1975), at least in forms occupying certain habitats and at very high latitudes. There are no indications that survival of the winter is a critical factor for any species at the North Swedish localities studied ($67^{\circ}50'N$, cf. III, IV). High population densities of some species can be present also in shallow water-bodies (unpublished data), which are thermically favourable during the warm season. However, the extremely long and cold winters of the Siberian forest-tundra appear to be troublesome even for the hardest species, and survival of larvae can only take place in certain habitats (KHARITONOV, 1975), illustrating the basic inability of odonate larvae to resist very low temperatures.

The third basic photoperiodic response is a promotion of development by long-day photoperiods. This well-known response,

which terminates the short-day diapause, can be further subdivided. One type (A) occurs in relatively early instars, where it contrasts with the short-day diapause (II, III, unpublished data on A. juncea). The rate of development is often slightly retarded (A. juncea, the penultimate instar of L. dubia), and during continued development at long-day photoperiods, this retardation becomes accentuated in one or more later instars (long-day diapause). The other type (B) is a high rate of development, often at, or close to the physiological limit of the species. It leads to emergence without interruption and is confined to instars above a certain, mainly genetically determined size (I-IV). This "final rush" in larval development normally requires a previous exposure to short-day photoperiods (autumn), and is thus preceded by a substantial increase in daylength (spring). This is demonstrated in the frequently observed reversal in response to artificial long-day photoperiods during late summer and autumn, from a delayed development (long-day diapause) to a rapid development (cf. CORBET, 1980; I, II, IV). It appears that both the magnitude of the increase and in what range of photoperiods the increase occurs is important (II, unpublished data on A. juncea). The latest developmental stages seem to react earlier and more readily to these changes in photoperiod than somewhat earlier ones (II). The exposure to low temperatures during the cold season, followed by high temperatures, cooperates with, or may partly replace the responses to the changes in photoperiod (LUTZ, 1974a; INGRAM, 1975). The sensitivity to photoperiod is often low after the winter (e.g. I-IV).

In the natural populations examined, the lower limit of the stages sensitive to long-day stimulation of development (type B) can be expressed as a critical size in the overwintering population (IV). Larvae that have overwintered in an instar above this size, develop rapidly and emerge during the following summer (I-IV). Smaller larvae ultimately enter long-day diapause, often already at the start of development during the spring, thus preparing for another overwintering in the larval stage. These different responses result in a separation of cohorts in the population.

This critical overwintering size is found to be a most important property, which does not seem to be related to the age of the larvae. It is a pivotal point in the seasonal regulation of emergence

and differs between species according to their phenology. Also different populations of the same species are genetically adapted to the local conditions by means of variations in the critical size (I, III, IV; NORLING, 1975).

The critical overwintering size may, to some extent, be modified in an appropriate fashion by environmental factors, e.g. temperature and photoperiod. In Aeshna (I; see also NORLING, 1975), where this effect is most conspicuous, it enables a population to adapt to the sometimes widely different conditions for growth in different water-bodies. That is, if low temperatures prevail, the critical size is increased.

The usefulness of the critical overwintering size model is likely to be restricted to species and populations where relatively few instars can precede emergence in one year. Here, the true "critical time" for the determination of the subsequent development may be the time in spring when the first ecdysis is prepared and performed. However, there are indications that the final course of development of some larvae, overwintering near the critical size, may not be determined until later (cf. experimental results in I; unpublished observations on A. cyanea). In SCHALLER's (1960, 1962) studies on A. cyanea in France, the latter part of the cohort separation in the mixed one and two year development displayed in his work, was similar to that in the South Swedish Aeshnas, but it started already during the same season as the eggs hatched. This situation is far from the critical overwintering size model, and at present difficult to explain. Probably, environmental factors were acting during a temporally dispersed and late "critical time" (cf. SCHALLER, 1960, Figs. 11, 12), which is contrary to SCHALLER's own conclusion.

Further refinement in seasonal regulation can be added by different effects of diapause on the winter population structure (cf. p. 5), and by different temperature responses during the spring (e.g. LUTZ, 1968) not demonstrated in this work. In the phenologically early species studied here (II-IV), the winter population structure was affected by a cohort separation, which anticipated the one at the critical overwintering size, and served to refine seasonal regulation within the gross framework of the critical size model (IV). In L. dubia (III), a different readiness to enter the short-day diapause at different developmental stages near the critical size appeared to cause the phenomenon.

According to a simple model, related to generation length, a species able to overwinter only in the larval stage can be expected to show certain trends in the pattern of life-cycle changes exhibited during a northward expansion of its range.

If the length of one generation is less than one year, but not short enough for a partial bivoltinism, each successive generation tends to overwinter in a more advanced stage than did the preceding one, and the population as a whole tends to overwinter in the latest stages permitted by seasonal regulation. Synchronization of emergence can be expected to be relatively good. When the generation length increases towards one whole year, the load on seasonal regulation decreases, and the winter tends to be spent in earlier instars. These two patterns probably caused the altitudinal differences in the winter population structure in Enallagma aspersum (HAGEN), reported from North Carolina by PAULSON & JENNER (1971).

When slightly more than one year is required to complete development, each generation tends to overwinter in successively earlier instars, until seasonal regulation has to restore the situation with a semivoltine generation. Such a population will spend the the final winter in a variety of instars, between the "critical size" and the latest instar permitted by seasonal regulation. Emergence can be expected to be poorly synchronized and without any sharp peak.

As the generation length continues to increase, a larger part of the population has to overwinter twice and consequently spend the winter before emergence in a late stage. The emergence pattern will show an early peak that represents the semivoltine individuals. Simultaneously with the increase in generation length (correctly: decrease in summer length), the critical overwintering size has to increase in order to maintain a proper seasonal regulation. This increase in critical size is likely to be accompanied by a shift in the intensity and/or location of the diapauses controlling the development, so as to permit a suitable winter population structure to be formed above the critical size despite shorter summers (cf. III, IV). Thus, at a generation length just below two years, there will be a strong tendency to show the spring species type of life-cycle. Good examples are Coenagrion hastulatum (CHARP.) at some places in southern Sweden (IV), Anax imperator LEACH (CORBET, 1957), and probably some Libellula species in North Carolina at higher altitudes (PAULSON & JENNER, 1971).

If the generation length is still more increased, the above cycle tends to be repeated. However, the larger part of the summer now necessarily occupied by the cold-sensitive period associated with reproduction produces a higher critical overwintering size, which increases the tendency to spend the last winter in the final instar in a general way: The number of alternative instars is reduced, and more larvae tend to accumulate in this instar as an alternative to a late emergence.

In an area, like southern Scandinavia, where short and often cool summers force an increasing numbers of species to adopt a two or multi year development, the "spring species pattern" thus becomes conspicuous, but the "summer species pattern" is still feasible for many species, in particular when an egg diapause is present (e.g. Aeshna; I). Owing to the low number of instars that in most species can be completed during one season, a well developed phenological separation often has to be associated with overwintering in the final instar, or not. However, overwintering in different intermolt stages of the final instar is an important contribution to phenological specialization (cf. I and III; NORLING, 1975).

At very high latitudes in Scandinavia, the short and cool summers becomes a major obstacle for the existence of Odonata. If other factors have not reached a limit before, the limit for a species is reached when the duration of the cold-sensitive period around reproduction equals that of the summer (III). Near this limit for dragonfly existence all species have to overwinter in the final instar, and the phenological variation between species is greatly reduced (III; NORLING, 1975; KHARITONOV, 1977, 1980).

A somewhat better utilization of the short summers by northern populations is achieved by overwintering slightly closer to metamorphosis than in southern populations (III, IV, unpublished data on A. juncea). For the same reason, an egg diapause is also of particular value at these latitudes, and puts the late-flying Aeshna species in a favourable position. It is doubtful to what extent an egg diapause has evolved in genera where it is normally absent, e.g. Coenagrion (IV) and Leucorrhinia (III). However, KHARITONOV (1977) reported egg overwintering in the three species examined by him in the Siberian forest-tundra: Somatochlora sahlbergi TRYBOM, Aeshna coerulea (STRÖM) and Coenagrion hylas (TRYBOM). The last mentioned

example is remarkable, and the egg diapause is probably the key to the successful colonization of this harsh area by this species. However, the successful adaptation to temperate climates is usually the result of the great evolutionary flexibility in the larval stage.

Evolutionary flexibility in the larval stage of Odonata is also found in their organs for aquatic respiration. The larvae of recent Odonata are well adapted to an aquatic life, except for a few, secondarily terrestrial forms (CORBET, 1962). The tracheal system with distinct, but in the water functionally closed spiracles suggests that the aquatic mode of life is a secondary one, although certainly one of very old age.

The different solutions to the problem of aquatic respiration in Odonata are, except the primitive general cutaneous respiration: the rectal gills in Anisoptera and Epiophlebiid Anisozygoptera; the caudal gills in Zygoptera, which also may have other functions; the retractable gill tufts in the archaic zygopteroid family Amphipterygidae, derived from the laminae subanales and protected by the caudal appendages (WATSON, 1966); and finally the lateral abdominal gills in the zygopteroid families Euphaeidae and Polythoridae (V, p. 4). This indicates that adaptation to aquatic life has differentiated early in the order. The following discussion is centered around the development of the lateral gills, as this gill type is also found in other plesiomorph orders, e.g. Ephemeroptera (cf. V, p. 35), and it was assumed by SNOODGRASS (1954) to be the most primitive organs for aquatic respiration developed in the Odonata.

The recent discovery of an Upper Carboniferous zygopteroid juvenile (KUKALOVA-PECK, pers. comm.) shows that at least gill-like lateral appendages really were present early in the history of Odonata. However, the caudal end of the specimen is missing, and nothing is therefore known about the caudal appendages. The fossil is at least an indication that aquatic habits might already have evolved at that time.

The primitive means of aquatic respiration certainly was a general cutaneous respiration. The frequently thin-walled abdominal pleural areas would have been a suitable part of the segment to evolve lateral gills as secondary invaginations. Also existing appendage anlagen in this, or other areas would have been useful precursors for gill development, and different types of gills may have evolved in parallel.

An attempt has been made by me to study the ontogeny and histology of the lateral gills in order to clarify their origin and to establish their possible function and adaptive significance in recent species. The results (V) have been discussed according to current hypotheses and the new interpretations of wing origin presented by KUKALOVA-PECK (1978). It was concluded that the facts accumulated during the study not necessarily are in conflict with any of the hypotheses discussed. The final interpretation of the gills depends on the segmental origin of the thoracic wings and how the embryonic pleuropodia have evolved. The view that the lateral gills represent styli or telopodite rests was by me regarded as slightly more likely than a serial homology with the thoracic wings or as an entirely secondary origin (V).

However, in a recent study on Machilid embryology, MACHIDA (1981) concluded that, contrary to current opinion, the pleuropodia are serially homologous with the abdominal styli, and not with the eversible vesicles. If this is correct, a telopodite or stylus origin of the odonate lateral gills is unlikely, as the embryonic gill anlagen do not appear to be serially homologous with the more ventrally placed pleuropodia. The possibility that the odonate lateral gills are entirely secondary structures, or that they may have a common origin with the more dorsally placed and probably "wing-derived" gills of Ephemeroptera, thus seems to be greater than assumed in my study (V).

If the lateral gills are of such a high age in the order, their evolutionary success has not been very great in Odonata, which contrasts with the situation in Ephemeroptera. In recent odonate species lateral gills are only present in a few rheophilic forms. Maybe these structures have been too vulnerable to evolve into effective gills in most habitats, and they are now surviving only in forms, which are confined to special and maybe intermittently oxygen deficient habitats.

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PHOTOPERIODIC RESPONSES AND LARVAL DEVELOPMENT IN LEUCORRHINIA DUBIA
(VAN DER LINDEN)(ANISOPTERA: LIBELLULIDAE): A COMPARISON BETWEEN
LARVAE FROM NORTHERN AND SOUTHERN SWEDEN

U. NORLING

Abstract

The larval development and photoperiodic responses of a population of L. dubia from north of the Arctic Circle were examined experimentally and compared with results from a South Swedish population. In both populations, larvae in the instars immediately preceding the final one show a photoperiodic reaction of the long-day type, and a conspicuous hibernation diapause is induced under short-day conditions. A long-day induced diapause starting in the early part of the final instar, and a short-day induced diapause at a slightly later stage, ensure that emergence is restricted to the spring, and that most larvae spend the winter before emergence in a suitably advanced stage within the final instar. The remaining development is stimulated by long days during the spring. The critical daylengths for these events differed by approximately 4-6 hours between the two populations, and northern larvae frequently showed short-day type responses at LD 21,5:2.5 and at continuous light (LL). Apparently photoperiod is no longer a sufficient source of temporal information in the northern study area, and the induction of hibernation diapause may have to take place under LL conditions, assisted by low temperatures. Even at LL, the long-day diapause in the final instar is weak in the northern population, and corresponds to an intermediate-day-length response in the southern population. Together with differences in the regulation and timing of the ecdysis into the final instar, this weak long-day diapause can explain why the final instar larvae in the northern population could overwinter in a more uniform and advanced state than in the southern population despite the shorter summers. Possibly the rate of development during the spring, before emergence, is more rapid in the northern population. An early, brief emergence period is believed essential for L. dubia in North Sweden where successful reproduction may be curtailed by short, cool summers.

INTRODUCTION

In temperate latitudes, photoperiod is one of the key factors in the timing of important recurring events in the life-cycle of many Odonata (CORBET, 1980). The interaction of the often complex photoperiodic responses and temperature permits a flexible adjustment of the life-cycle to the variable conditions that may occur also within a small and climatically homogeneous area (NORLING, 1975). The photoperiodic responses of populations from different latitudes are known to be genetically adapted to the average local conditions in many other insects, e.g. by different critical daylengths¹⁾ (cf. BECK, 1980). In Odonata, the extent and expression of such a genetically based variation is little known.

Leucorrhinia dubia is a common and often dominant species at bog-pools and other similar, usually acid waters in Sweden. It appears to be as successful in the harsh climate of the northernmost spruce forest area as in southern Sweden. The seasonal regulation of a population in the latter area (Fjällmossen) was presented in an earlier communication (NORLING, 1976), in which emphasis was placed on the responses of larvae in the final larval instar to photoperiod and temperature. For comparison, some of these results are included here, and some unpublished data from this population have been added.

The purpose of the present investigation was to examine and compare the photoperiodic responses of populations from climatically different areas. The implications of the results as related to adaptations to the climate of the respective areas are considered.

MATERIAL AND METHODS

Most of the northern larvae were collected in the Sappisaasi area, 67°50'N, 21°40'E, north of Vittangi, in three water-bodies at approximately 335, 375 and 400 m above mean sea level. They were all situated in the boreal spruce forest area. Samples were taken on 16-18 June and 21-22 July to obtain information on the life-histories of the species. Collection dates of larvae used in the experiments are shown in Table I.

1) Critical daylength, or critical photoperiod, is the point of transition between two different types of response, e.g. high and low incidences of diapause (BECK, 1980). When the response to photoperiod is partly graded, as frequently in Odonata (e.g. NORLING, 1976), a critical daylength may be difficult to define exactly.

Material for experiments were also collected in the Abisko area, 68°20'N, 18°50'E, about 380m amsl, in the montane birch forest. All material of northern origin was subsequently treated as "one" population (N). The Abisko material was evenly dispersed among the experimental groups.

Material of southern origin was principally obtained from Fjällmossen, 58°42'N, 16°31'E, 84m amsl; but limited experiments were also performed on larvae from Holmeja, 55°33'N, 13°17'E (Table I). Reference to the southern (S) population normally means the Fjällmossen population.

Collections were made with a net (diameter about 0.3m) with 1x1mm mesh size, separately supplemented with a small net (diameter 120mm) with 0.2x0.7mm mesh size sufficient to retain the smallest specimens. The net contents were either washed out in plastic trays or were placed on a plastic sheet for rapid examination for larger larvae.

Difficulty was experienced in separating L. dubia and L. rubicunda (L.). The latter species was common on the N localities and was the only other libellulid to be found there; however, L. rubicunda was scarce at the S study site. The abdominal pigment pattern readily served to separate larvae with a head width exceeding 1.7mm (i.e., the last 6 or 7 instars). Larvae with a head width of less than 1.0 mm (i.e. from the prolarva, which is equivalent to the first instar, to instar 4 or 5) could not be identified to species.

The head width of the last five instars was measured according to NORLING (1971), while smaller specimens, usually preserved in alcohol, were measured using an ocular grid in a microscope.

Experiments involved mainly larvae in the last five instars, referred to as F (final), F-1 (penultimate) and etc. to F-4. The treatment between collection and the start of the experiments is presented in Table I. For larvae stored at 4°C, the change to experimental temperatures was allowed to take at least 5 hours. The experiments were performed in insulated, light-proof boxes, each supplied with a 4W white fluorescent lamp, shaded to produce an even illumination of 100-200 lux at the bottom. The larvae were kept in separate compartments in large trays provided with a moderate flow of constant-temperature water pumped from a central external source. Temperature fluctuations rarely exceeded $\pm 0.5^{\circ}\text{C}$.

The responses to photoperiod were studied mainly at 20°C: Both populations at LD 13:11 (13hr light: 11hr dark), 16:8, 19.3:4.7 and LL (continuous light); the S population at LD 17.7:6.3; and the N population at LD 21.5:2.5. At 15°C, the responses were examined at LD 13:11 (S population), 16:8, 19.3:4.7 and LL (both populations). No more than eight experimental groups could be run simultaneously because of equipment limitations. Experiments were also limited by lack of space and the amount of material available. Each day the larvae were checked for ecdyses and were fed ad libitum with Tubifex. Larvae undergoing diapause were checked at 2 or 3 day intervals.

It was necessary to transfer F-1 larvae from one condition to another to study the responses in the F instar at photoperiod/temperature combinations unsuitable for development in earlier instars. The transfer was made at a time near ecdysis, which was judged by eye development (ELLER, 1963; NORLING, 1976). The remaining duration of the F-1 stadium was considered to be determined by the original photoperiod. A change of photoperiod probably does not affect development during the final stages of the moulting cycle. At a transfer between photoperiods at the same temperature, the total duration of the F-1 stadium at the original photoperiod was thus estimated by adding this remaining duration to the period previously spent in the instar; however, at a transfer from 20°C to 15°C, the remaining duration was halved before being added to the initial period, spent at 20°C. The rate of non-diapause development at 15°C is approximately half that at 20°C (Fig. 4h, 1; NORLING, 1976).

The F instar was arbitrarily subdivided into five phases, defined by eye and wing development (see NORLING, 1976). In the experiments, the duration of these phases was estimated by regular observations.

RESULTS

Life-history

The S population usually had a 3-year life-cycle. Emergence took place in early June, oviposition occurred mainly in June and early July, and eggs began to hatch during the first half of July (NORLING, 1976),

and continued to hatch into August. The material from the N localities was insufficient for a reliable estimate of the duration of the life-cycle; however, it appeared to be longer than three years.

Two aspects of the pattern of development were similar in the N and S populations. The first winter after oviposition was spent in early larval instars (Fig. 2), and the winter before emergence was spent in the F instar. However, the stages of development during the first winter are less well known for the N population than for the S population. There remained the possibility that part of the N population overwintered in the egg stage, although a cold resistant egg stage is unknown within the genus Leucorrhinia. Even moderately small larvae could be difficult to find at the N localities, in particular during the spring.

In both N and S populations, larvae accumulated in the F instar during the summer. Before winter, development usually continued up to phase 4 (NORLING, 1976), a stage not far from the initiation of the moulting cycle, and the winter was spent here. Larvae in the F instar overwintered in a more uniform and advanced stage in the N population than in the S population (Fig. 3). Shortly before the start of emergence, the difference in synchrony between the two populations appeared to be greater than during winter, which indicated a greater variation in the rate of development among the overwintered F instar larvae at the S locality.

On 16-18 June 1972 emergence had not yet started at Sappisaasi (N), but the advanced state of development of the F instar larvae observed on this occasion, together with the warm weather, indicated that emergence probably took place during the last 10 days of June, most likely with the last individuals emerging in early July at one of the localities. Emergence was thus no more than three weeks later than for the S population. On 13 June 1973, development in the F instar had barely started, indicating that emergence during 1973 may have started about one week later than in 1972. In the S area no difference in the start of emergence was detected between the two years.

A large number of F-1 instar larvae overwintered at all the N localities, which was not the situation at the S locality (Table II). The low frequency of F-1 instars found during the winter 1972-73 could be traced directly to the separation between the two year-classes that

hatched during 1970 and 1971. During 1973, the separation between the 1971 and 1972 year-classes disappeared early in the summer. Some larvae of the 1972 hatch certainly reached the F-3 instar by early July, and both year-classes are thus represented in the late summer samples shown in Table II. During late summer and autumn the frequency of F-1 instars decreased, indicating that a secondary cohort separation was splitting the previous continuum of instars. It appeared that growth was restricted in the F-3 and F-2 instars during this time. Collection at other places indicated that a low number of overwintering F-1 instar larvae is a common feature of populations in S Sweden.

Shortly before the start of emergence, the larvae that were in the F-1 instar were closer to ecdysis in the N population than in the S one, judged from eye development, although all these larvae had spent the winter in this instar. The start of the entry into the F instar at the N localities was probably less delayed than at the S locality (cf. NORLING, 1976); however, moulting did not appear to take place before the start of emergence in any of the populations, not even in the F-3 and F-2 instars.

Photoperiodic responses in the F-4 to F-1 instars

In the F-4 to F-1 instars the photoperiodic reaction was of the long-day type. The typical response to short-day photoperiods (daylengths below the critical value for the population in question) was a distinctly prolonged development, a diapause (diapause is here defined as a prolongation of development, that is not directly caused by adverse conditions; cf. BECK, 1980). In the November experiments (e.g. Fig. 4i) the first ecdyses were delayed as a result of a previously induced diapause; however, larvae that had spent a sufficient time at winter temperatures usually moulted once soon after the start of the experiments, and then re-entered diapause (Fig. 4a, b, j).

Long-day photoperiods promoted development and the larvae rapidly entered the F instar (Fig. 4h, l). Development of the southern F-1 instar larvae was slightly slower (22%) at long-day photoperiods (Fig. 5a, b) than at short-day or intermediate (near-critical) photoperiods if the

pronounced short-day arrest failed (Fig. 5c; NORLING, 1976).

The critical photoperiod observed in the laboratory differed greatly between the populations. In the S population LD 13:11 produced a short-day response whereas LD 19.3:4.7 and LL produced long-day responses (Fig. 4j, h), irrespective of season. LD 16:8 produced a distinct short-day response during late summer (Fig. 4f), and LD 17.7:6.3 produced a long-day response during winter experiments only. LD 16:8 during winter and 17.7:6.3 during late summer produced varied responses (Fig. 4g, k), indicating proximity to the critical photoperiod. In an experiment started on 30 November 1973 it was also noted that LD 16:8 did not delay the first ecdysis, although LD 13:11 did so. Overall, the winter experiments at LD 16:8 produced a response comparable to that of a delayed effect of short days.

Development of the N population was prolonged at LD 13:11 and up to LD 19.3:4.7 (Fig. 4a, b, i). The only experiment performed at LD 21.5:2.5 produced varied responses (Fig. 4c), that appeared somewhat closer to a long-day response than the southern LD 17.7:6.3 late summer group (Fig. 4g). Even at LL the response of the N population was not completely of the long-day type: varied responses occurred regularly in the F-1 instar (Figs. 4d, 5h). Nine out of the 35 larvae (26%), which were in the F-5 to F-3 instars at the beginning of the experiments, later showed a distinct prolongation of the development in the F-1 instar (duration more than 50 days) that was comparable to a short-day response. Slightly prolonged development was shown by three or four specimens; however, the majority of the larvae, including all those introduced in experimental conditions in the F-2 instar, showed a rapid development in the F-1 instar that was comparable to that of southern non-diapausing larvae at short-day or intermediate photoperiods (Fig. 5c, g, h). Also, the duration of the F-2 stadium appeared slightly prolonged at LL (Fig. 5i) when compared to the S population at LD 19.3:4.7 and LL (Fig. 5d, e). The only similar responses in larvae of S origin occurred at LD 17.7:6.3 in late summer (Figs. 4g, 5f). Generally, the LL experiments, like the LD 16:8 winter experiments with the S larvae, showed a delay in the onset of the short-day response (Fig. 4d, k). The small group of N larvae at LL, 15°C (Fig. 4e) apparently showed a typical short-day response in both the F-2 and F-1 instars (compare the S population at

15°C, Fig. 4l-n). Similar results at LL, 15°C was obtained during the 1977 experiments.

As noted earlier (NORLING, 1976) larvae from the S population were less susceptible to the short-day induced developmental arrest in the F-1 instar than in the immediately preceding instars (Fig. 4f, j, n). If the earlier instars showed a variable response, then the F-1 stadium was invariably of short duration (Fig. 4g, k, m), which may indicate a shorter critical daylength acting in the F-1 larvae. In the N population the opposite occurred. The larvae appeared more prone to display the typical short-day response in the F-1 instar than in the preceding instars (Figs. 4c, d, 5h, i).

Photoperiodic responses in the final instar

Larvae entering the F instar during the experiments showed prolonged development in this instar unless the daylength was increased substantially (NORLING, 1976). The rate of development in phases 1 to 4 varied and was dependent on photoperiod and temperature (Figs. 6, 7). The duration of phase 5, which is a minimum estimate of the time between apolysis and emergence (cf. SCHALLER, 1960, and NORLING, 1976), was short and appeared to be dependent on temperature only. Phase 5 usually took 10-11 days at 20°C and 19-22 days at 15°C. The change of the duration of phase 5 in response to temperature is similar to that observed for the duration of earlier stadia (NORLING, 1976; cf. Fig. 4), and probably is characteristic of rapid, or "normal", larval development.

The pattern of responses of the N population was generally similar to that of the S population (Fig. 6). At short-day photoperiods, the first three phases showed a weakly or moderately reduced rate of development, and the temperature response was approximately normal. At long-day photoperiods the reduction of the developmental rate was greater, the difference increasing from phase 1 to 3. In phases 2 and 3, the sensitivity to different photoperiods increased with temperature, partly producing an inverse temperature response at long-day photoperiods. The slowest development was generally found in phase 4, the duration of which varied relatively little between the groups.

Generally, development was faster in larvae of northern origin than in those from the S locality. Except in the short-day, 20°C groups, the difference between the populations was particularly great in phases 2 and 3, i.e. in the stages where the difference in response to photoperiod within each population was at maximum. The "critical photoperiod" for the transition from the short-day to the long-day response shown in Fig. 6 differs greatly between the two populations (Fig. 7), irrespective of how the comparison is made.

Experiments using northern F instar larvae that were held under cold conditions were carried out on four occasions. Most of the results are shown in Fig. 8, where also some southern material is included; however, for more complete results for the S population, see NORLING (1976). All the larvae from both populations developed rapidly at LL, 20°C, after transfer from cold storage in the dark. LD 13:11 produced a distinctly prolonged development in larvae that were in early developmental phases when stored, whereas this photoperiod had a less pronounced effect on larvae in more advanced phases. The response to LD 13:11 of the N population was similar to, but possibly less intense than that of the S population. The N population showed a response at LD 19,3:4.7 that was similar to the response shown at LD 13:11, whereas the S population developed at a rate closer to that at LL.

The experiments indicated that a seasonal change in response to photoperiod also occurred. As was found by NORLING (1976), the development was slightly slower in an early winter experiment (10 Nov. 1972) than in a later one (1 Feb. 1973), especially at short-day photoperiods. In the somewhat unusual 21 Feb. 1975 and 22 Sept. 1977 experiments (see pretreatment in Table I) an unusually high rate of development was shown; however, one individual (n= 6) at LL, 15°C, did enter diapause in the 22 Sept. experiment.

The most advanced N specimens (eye rim position 0.7-0.9 in Fig. 8) usually emerged only 1-2 days later at short-day photoperiods than at LL. An examination of wing and eye development data obtained from the 10 Nov. 1972 experiment indicated that the difference was caused by a shortened duration of phase 5 at LL (i.e. 9 days at LL compared to 10-11 days at LD 13:11 and 19.3:4.7). This effect on phase 5 was confirmed in the 21 Feb 1975, 22 Sept. 1977 (N material) and 17 Oct. 1977 (S material) experiments.

DISCUSSION

Overall, the photoperiodic responses of L. dubia larvae are considered to regulate development as follows (NORLING, 1976). Smaller larvae grow only in long-day conditions, when temperatures are suitable. As the daylength decreases during late summer, preparation for overwintering starts by induction of diapause. Adult phenology is primarily determined by the responses to photoperiod and temperature during the F stadium. Larvae that reach the F instar early during the summer develop slowly because of the effects of long days and high temperatures. During late summer, when the days are shorter, the rate of development increases in the early part of the F stadium, and the larvae start to accumulate in phase 4 (near the initiation of the moulting cycle), whereafter development appear to cease (hibernation diapause). Late entries into the F instar overwinter in an early developmental phase, and during the next spring they emerge somewhat later than the main group. During the spring, long days usually stimulate development in the overwintering instars, assisted by the effects of previous exposure to low temperatures. Emergence is normally restricted to larvae that have overwintered in the F instar.

Compared to populations in S Sweden, the N population of L. dubia experiences shorter, usually cooler summers, longer, more severe winters, less predictable weather conditions, and extreme natural photoperiods (Fig. 1; WALLÉN, 1965). The short summer of high latitudes is of particular importance as it restricts the amount of development that can take place within one season to a small fraction of the life-cycle. Survival of the long winters must occur in a wide variety of developmental stages, and periods of cold sensitivity must be of the shortest possible duration. L. dubia can overwinter as larvae in at least the third to F (13-14th) instar, but certainly not as adults or prolarvae (first instars), and probably not as eggs. Then, the following sequence of events must be short enough to occur within one summer: - 1. completion of larval development and emergence; - 2. adult maturation, mating and oviposition; - 3. embryonic development and hatching of eggs; - 4. development as far as to a larval stage capable of overwintering.

In the N population, the compression of the summer season has brought the shortening of the first and last events to, or near, its limit. Most larvae spend the winter before emergence in the most advanced stage possible (Fig. 3), and young larvae probably overwinter in the third and fourth instars (Fig. 2). This at least appeared to be the situation in 1972, when temperatures in the N area were exceptionally high during late June and early July, i.e. during maturation and egg development.

The adult and the egg stages were not examined in the present study; therefore, their possible contribution to adaptations to subarctic conditions is unknown. STEINER (1948) in Germany and PAJUNEN (1962) in Finland give adult maturation periods of 14 and 10-15 days, respectively, for females, and slightly less for males. Maturation can be shortened to about one week during very favourable weather (PAJUNEN, 1962). The average length of the male reproductive period was estimated to be 17 days (PAJUNEN, 1962). PRENN (1930) in Austria reported a 3 week duration of the egg stage, but egg development in L. dubia is probably strongly temperature dependent as in the North American L. intacta (HAGEN) (DEACON, 1975).

Tentatively, the minimum time from emergence of adults to hatching of eggs under "normal summer" conditions is about 30-35 days, which approximately agrees with the observations in the present study. However, periods of cold and bad weather prolong the maturation period and egg development, restrict the reproductive activities and increase the mortality. In the N area, such periods are often prominent, and during a cool summer, successful reproduction may thus be at stake. Also during relatively normal years, the period of successful reproduction for a substantial proportion of the northern adult population might be curtailed by the onset of cold conditions in the way that part of their offspring does not reach a resistant stage before winter. Unless an egg diapause is present, even a slight delay of the emergence may thus decrease the expected reproductive success significantly in the N area.

A conspicuous feature of the light conditions in the N area is the long period of continuous daylight around the summer solstice (Fig.1). For most insects the effective length of the day (photophase) probably

includes the periods of Civil Twilight (BECK, 1980). If this is also true for L. dubia under the conditions of the northern summer, the population at Sappisaasi experiences continuous light for a period of about 100 days, extending into early August. Accordingly, long critical daylengths are expected in this population. In fact, even LL frequently failed to produce a typical long-day response.

In the F-2 and F-1 instars, a typical hibernation diapause was noted at LL, 15°C (Fig. 4e). At LL, 20°C, this response became restricted to the F-1 instar, and it occurred only among larvae that reached this instar two or more ecdyses after "winter" (Fig. 5h). These short-day responses at LL appear to be connected with an extremely long critical daylength, which interacts with temperature. Apparently photoperiod alone is an insufficient source of temporal information in this population, at least for the induction of hibernation diapause in the above mentioned instars. The delay in the onset of diapause at 20°C, and perhaps the effect of temperature, may be explained by a slow increase in critical daylength after the overwintering. During winter, the critical daylength appears to be depressed by the action of short days and low temperatures (cf. p. 7; SAWCHYN, 1972; DEACON, 1975; NORLING, 1976).

Larval diapause is hormonally regulated, and normally delays the events that trigger the moulting cycle (BECK, 1980). The latter consists of apolysis, secretion of new cuticle, and ecdysis. Once started, the moulting cycle is considered to be beyond strict hormonal control (LOCKE, 1976). During prolonged exposure to temperatures below the threshold for ecdysis, tentatively near 7°C, mortality is high in larvae caught between apolysis and ecdysis, whereas larvae in the early phase of the stadium survive (personal observations on Aeshna species; see also note under Table I). A hibernation diapause ensures that the winter is encountered in an early interecdysis stage, and the initiation of diapause should start at a time when the moulting cycle, including its hormonal inception, can still be completed in larvae that just have escaped diapause induction.

In the N area, there is a definitive, often sudden temperature drop

during September, after which no development can take place. If apolysis occurs about half-way through the stadium, as in the F-1 instar of Aeshna cyanea (MULL.) (SCHALLER, 1960), the moulting cycle in the F-1 instar of L. dubia can be estimated to require 3 weeks at 15°C, and more at lower temperatures. Then, it does not seem unlikely that diapause inducing conditions have to start at continuous light to ensure that the winter is encountered in a suitable state. In the S population, diapause in the F-3 and F-2 instars appears to start during mid-August (cf. Figs. 1 and 4f, h), which is approximately equivalent to mid-July in the N area (Fig. 1).

Also some responses in northern F instar larvae may be related to a critical photoperiod close to LL. The rate of prolonged development (long-day diapause) at LL was similar to that at near-critical photoperiods in the S population (Fig. 7). At any rate, this weak long-day diapause is appropriate in the N area, where it enables most larvae to reach phase 4 before winter despite extreme long-day conditions and a short summer. An early entry into the F instar in the N area is, in this respect, comparable to a late summer entry in the S area. A weak summer diapause of the northern type in South Sweden might produce some autumn emergence.

At short-day photoperiods and 20°C, the difference in F instar development between the populations was minor; however, at 15°C, a temperature more usual during short-day conditions in the field, the difference was greater, except in phase 4 (Fig. 6). The difference may be fundamental, giving the N population a higher potential for synchronization in phase 4.

Exposure to shorter days during autumn sensitizes the larvae in the F instar to long-day stimulation of development (NORLING, 1976). Probably, low temperatures can have similar effects as short days in this respect (INGRAM, 1975). Prolonged exposure to low temperatures also increases the ability of the larvae to begin rapid development as soon as the temperature becomes favourable, even at short-day photoperiods. After a long period of cold storage of F instar larvae in phase 4, the hormonal signal to start the moulting cycle is probably triggered almost immediately upon return to higher temperature.

Less advanced F instar larvae react conspicuously to photoperiod and show critical daylengths roughly similar to those for other developmental events (Fig. 8; NORLING, 1976, Fig. 5). Overall, the most rapid development in cold treated F instar larvae was observed in the N population, which may indicate an increased capacity of these larvae to start and complete development rapidly during the spring.

In the N population, the F-1 instar showed a greater propensity to enter diapause than the earlier instars, which possibly is related to a longer duration of the moulting cycle in the F-1 instar. However, in the S population the situation was reversed. The F-1 instar appeared to enter diapause later than the F-3 and F-2 instars. This difference between the populations is probably related to seasonal regulation and the climatic differences between the study areas.

In the N area, an early diapause in the F-1 instar not only ensures a high survival of the winter, but it also produces a certain accumulation of larvae in this instar, and late entries into the F instar are largely prevented. The result is a concentration of the entries into the F instar to the early part of the season. During the rest of the season, the responses of the F instar ensures a good synchronization in phase 4, and a frequency gap in the early part of the F stadium is produced. In the S population, the situation is in reality similar, but the different events are displaced towards earlier developmental stages, which reflects the longer summers. The earliest induction of hibernation diapause, and the accumulation associated with it, takes place in the F-3 and F-2 instars, while the responses in the F-1 instar foreshadows those in the early F stadium. Rapid development in the F-1 instar at short-day photoperiods to some extent occurs, producing a frequency minimum in this instar during winter, and a very weak long-day diapause produces a certain delay of the entry into the F instar during early summer. Entries into the F instar thus tend to become concentrated towards the later part of the summer. There is also, in this population, a generally slower development (stronger diapause) in the early part of the F stadium. Therefore, the last winter is spent in a less uniform and less advanced state of development than in the N population. The frequency minimum, which is entirely confined to the early F

stadium in the N population, is here visible as a separation between cohorts.

The longer summers in the S area makes an extremely early and brief emergence period less important, and larvae overwintering in the F-1 instar can sometimes be stimulated by long-day conditions during the spring, quickly pass through the F instar, and emerge after the main group (NORLING, 1976, Fig. 2; cf. also PRENN, 1930). The F-1 instar is thus a critical stage in this area, and the number of larvae overwintering in this instar may significantly affect the phenology of the population.

The whole complex of responses involved in seasonal regulation, including the cohort separation, is, through selection, affected by the interaction of 1) the expected reproductive success as a function of the date of emergence, 2) the likelihood for winter survival as a function of the timing of the induction of hibernation diapause, and 3) mortality as a function of the time spent in the larval stage.

In the N population, the observed physiological properties aim at the earliest possible emergence, at the expense of a shorter developmental time. In the S area, a shortening of the larval life at the single expense of early emergence and synchronization occurs when emergence takes place after an overwintering in the F-1 instar. It appears that also a reduced likelihood for winter survival, associated with late entries into the F instar, may be involved here. Depending on the fate of overwintering F-1 instars, a late entry either improve synchrony, or shorten the larval life at a relatively slight cost of synchronization.

The only other European Leucorrhinia species that reach subarctic areas is the closely related rubicunda. Observations in the field suggest that this species grows more rapidly (Fig. 2), but otherwise has a life-cycle similar to that of dubia, and it has the same kind of synchronization within the F stadium. However, rubicunda emerges earlier (PAJUNEN, 1962), and may react more rapidly to the increase in temperature during the spring. The earlier emergence may explain its greater success in such climatically harsh areas as the Scandinavian

montane birch forest 70-80 km north of Vittangi, at Abisko and in northern Norway (personal observations).

The North American L. intacta, which has been studied in an area with warmer summers than in Scandinavia (Ontario, Canada; DEACON, 1975), is another interesting comparison. It is early, as are the European Leucorrhinia species, but an early, synchronized emergence appears less important than in the dubia populations. The F instar holds a less unique position in the fast-growing, univoltine L. intacta, and the winter before emergence is spent in several instars. For overwintering, the F instar is reached only during the late summer and autumn because of a long-day induced diapause in the F-1 instar. The development during the early part of the F stadium at short-day photoperiods is significantly slower than in dubia, and the winter is therefore spent in approximately phase 1 and 2 (DEACON, 1975, and pers. comm.)

The pattern of development in the L. intacta population represents a continuation of the climatically related trend seen between the N and S populations of L. dubia. Responses, which are entirely, or mainly, restricted to the F instar in the latter populations, are here largely extended to earlier instars, and overwintering F instar larvae are in the very beginning of the stadium. With the tropical origin of Odonata born in mind, the life-cycle pattern of L. intacta must be regarded as the most primitive one. The patterns of development in the northern species and populations are secondary modifications, suitable for survival near the northern limit for dragonfly existence, or for phenological specialization.

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Table I. Dates of collection, subsequent treatment, and timing of experiments for material used in the present investigation. Within each area (N= northern; S= southern) the place of collection is shown in parenthesis (V= Sappisaasi, Vittangi; Ab= Abisko). For the northern material, where larvae from different places were mixed in the experiments, the number of larvae used experimentally is also given.

	Collection date	Treatment before experiments	Start of experiments
<u>N</u>	16-17 Sept. 1972 (V; n= 96)	0-4°C; DD ¹⁾	10 Nov. 1972 1 Feb. 1973
	13 June 1973 (V; n= 33)	4°C; DD	17 Jan 1974
	17 June 1973 (Ab; n=13)		
	17 July 1974 (V; n= 40)	"Chilled" until 25 July ²⁾	14 Jan 1975 21 Feb 1975
	21 July 1974 (Ab; n= 7)		
	July 1977 (Ab; n= 55) ³⁾	4°C; DD	22 Sept. 1977
<u>S</u>	12 Oct. 1972 (Fjällmossen)	0-4°C; DD	5 Dec. 1972
	26+29 Oct. 1973 (--"--)	4°C; DD	30 Nov. 1973 25 Jan. 1974
	8 Aug. 1974 (--"--)	Approx. natural photoperiod and temp. to 15 Aug. ²⁾	12-15 Aug. 1974 3 March 1975
(S')	14 Oct. 1977 (Holmeja)	4°C; DD	17 Oct. 1977

1) In reality occasionally interrupted darkness.

2) Thereafter placed in an environmental chamber with LD 6:18 and 14°C during the photophase and 7°C during the scotophase (for induction of diapause); transfer to 4°C and DD during November.

3) Kindly collected by Mr Olle Hammarstedt. Almost all specimens that were preparing for ecdysis died during the cold storage, or shortly afterwards, whereas the larvae that had not reached apolysis at the time of collection remained healthy.

Table II. Number of larvae in the four last instars at different collection dates.

Date	Instar			
	F-3	F-2	F-1	F
S population, main site				
4 May 1972				
12 Oct.				
17 Apr. 1973	61	51	2	65
9 May				
22 July 1973	20	15	17	11
4 Aug.	32	24	16	18
17 Aug.	22	15	5	16
31 Aug.	14	16	8	23
14 Sept.	12	18	4	15
26+29 Oct. 1973				
2 May 1974	42	87	8	99
S population, pool adjacent to main site				
4 May 1972				
12 Oct.	24	13	2	26
26+29 Oct. 1973				
S population, total overwintering (12 Oct. - 9 May)				
	<u>127</u>	<u>151</u>	<u>12</u>	<u>190</u>
N population				
16-18 June 1972	25	48	65	29
16-17 Sept.	8	18	21	55
13+17 June 1973	37	19	15	17
Total overwintering	<u>70</u>	<u>85</u>	<u>101</u>	<u>101</u>

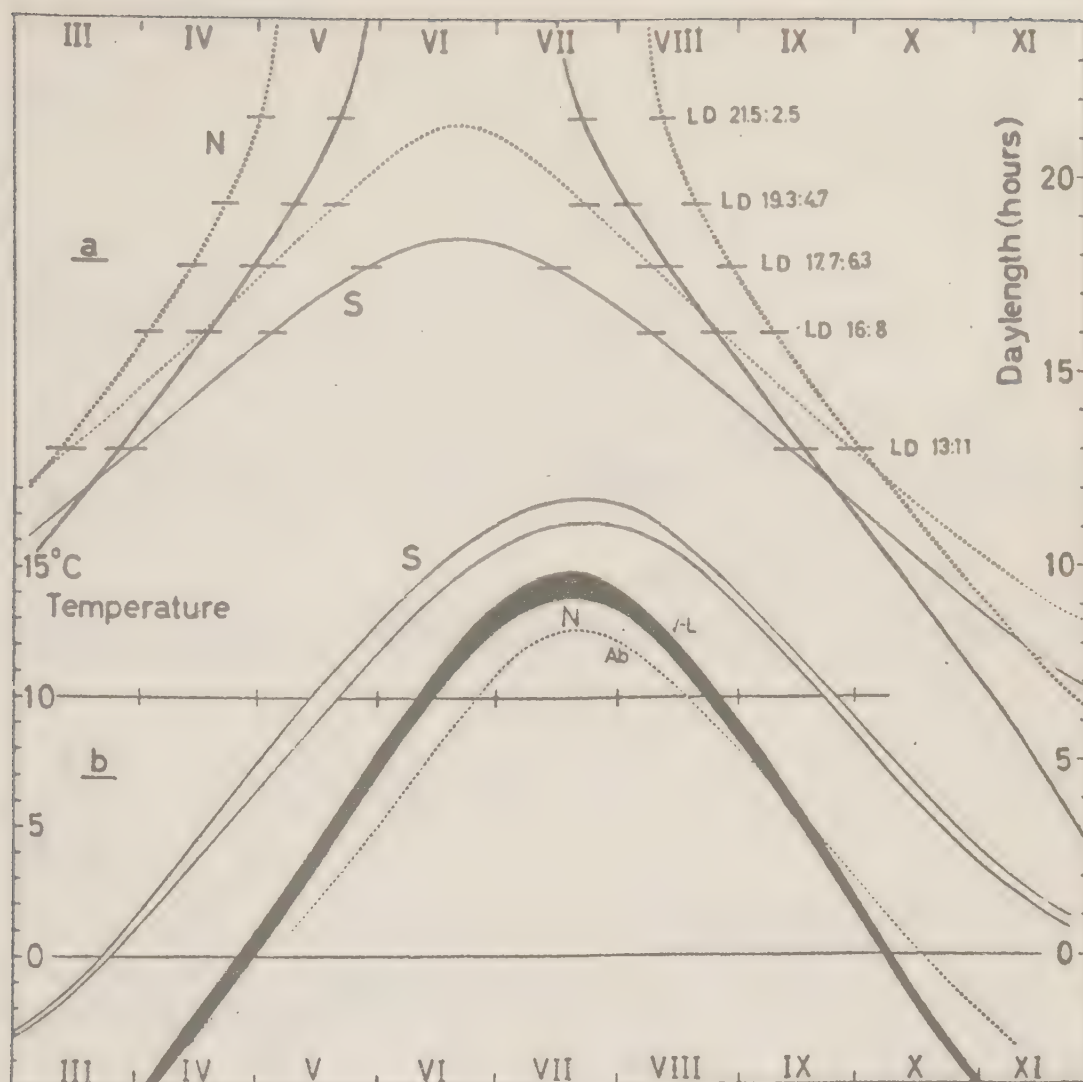


Fig. 1. Daylengths and mean air temperatures in the southern (S) and northern (N) study areas. The months from March to November are indicated on the abscissa. - (a) Daylength, calculated from KALAJA (1958) for $58^{\circ}42'N$ and $67^{\circ}50'N$. - Solid lines: sunrise to sunset; - Broken lines: the same, with the periods of Civil Twilight added. Photoperiods used in the experiments are indicated on the curves (LD= light: dark period). - (b) Air temperature, drawn from monthly reference values calculated for the period 1931 to 1960 (S.M.H.I., 1973). The solid curves give the range between two stations within 30 km of the study sites in each area: Norrköping - Nyköping (S; white curve) and Vittangi - Lannavaara (N: V-L; black curve). The broken line (N: Ab) represents Abisko.

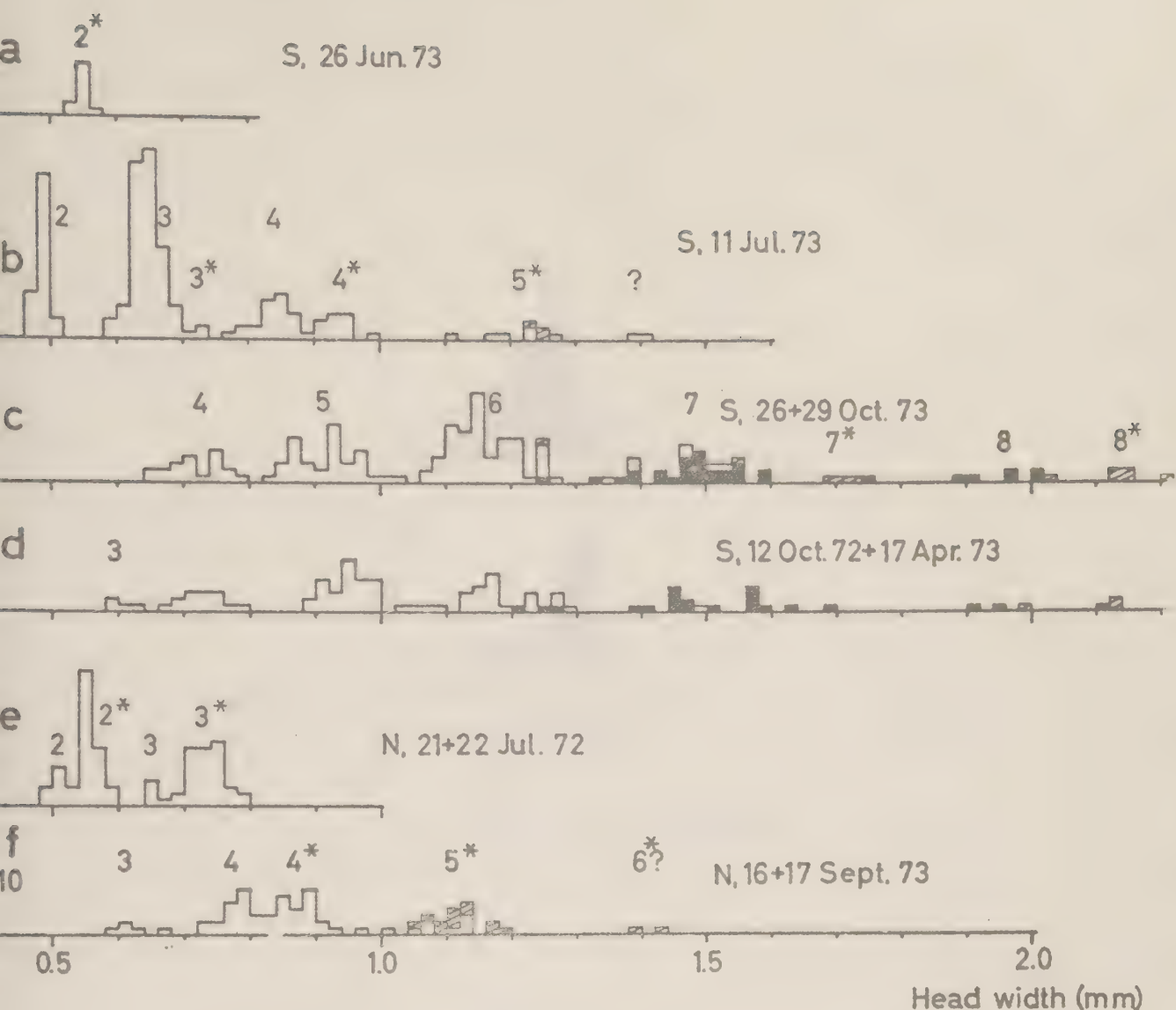


Fig. 2. Head width size-class frequency histograms of *Leucorrhinia* larvae from the S and N study sites, collected soon after the start of hatching (a, b, e), and after completion of their first growth season (c, d, f). Larvae that could not be identified are shown in white, *L. dubia* in black, and *L. rubicunda* with oblique lines. In (c) and (d), the largest of the fast-growing *rubicunda* larvae are too large to be included. The frequency peaks represent separate instars and are assigned instar numbers, counted from the egg stage. The instars suspected to be *rubicunda* are indicated by an asterisc. Because of the collection methods, the samples are strongly biased towards the larger sizes.

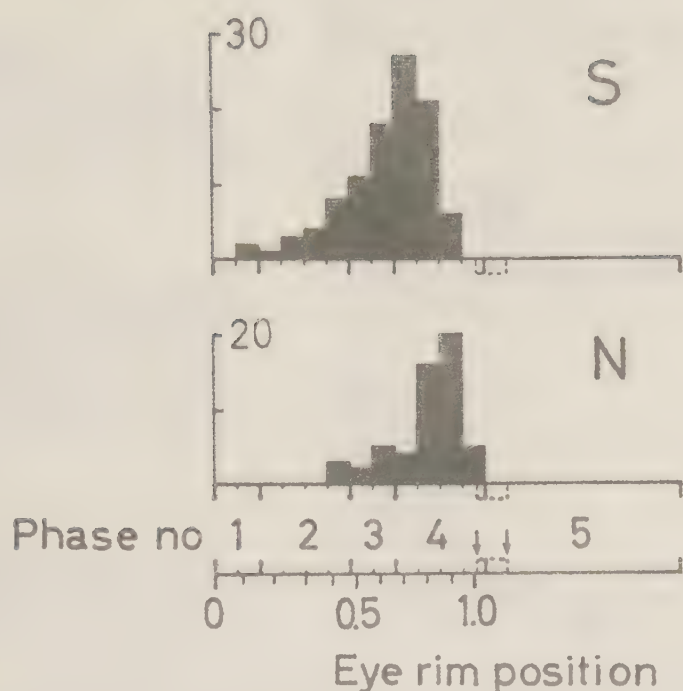


Fig. 3. Frequency histograms showing the distribution of larvae within the F stadium during the winter. The base line represents the development during the F stadium at rapid (stimulated) growth, from ecdysis (left) to emergence (right), which takes about 25 days at 20°C. The developmental phases used previously (NORLING, 1976) are indicated and have been further subdivided. The relative posterior position of the eye rim is shown below the histograms (cf. Fig. 8). The variation in eye rim position at the start of phase 5, which is defined by wing development (corrugation of costa), is indicated by arrows. - S population: Collection on 12 Oct. 1972 (n= 16), 17 Apr. 1973 (n= 5) and 26+29 Oct. 1973 (n= 80). - N population: Collection on 16-17 Sept. 1973.

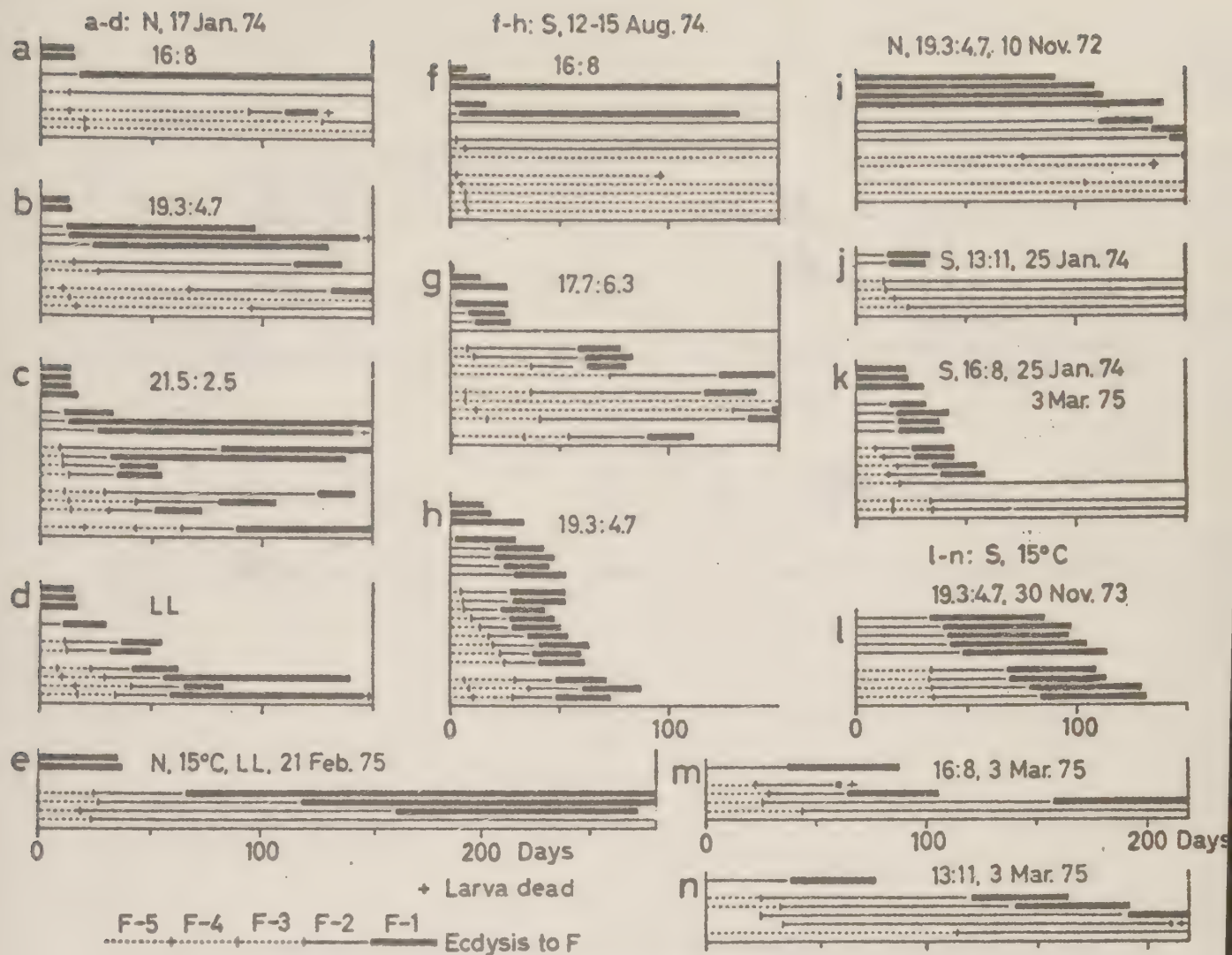


Fig. 4. Individual development of larvae up to the entry into the F instar, showing the different responses to various experimental conditions. Where possible, parallel experimental groups have been selected. The instars are indicated according to the legend, and the ecdyses are shown as transverse bars, except between the F-2 and F-1 instars. Origin of larvae (S or N population), date on which experiment was begun, and photoperiod (light: dark periods) are shown. The temperature was 20°C unless otherwise stated (i.e. in e, l-n). The vertical zero line indicates the start of the experiment.

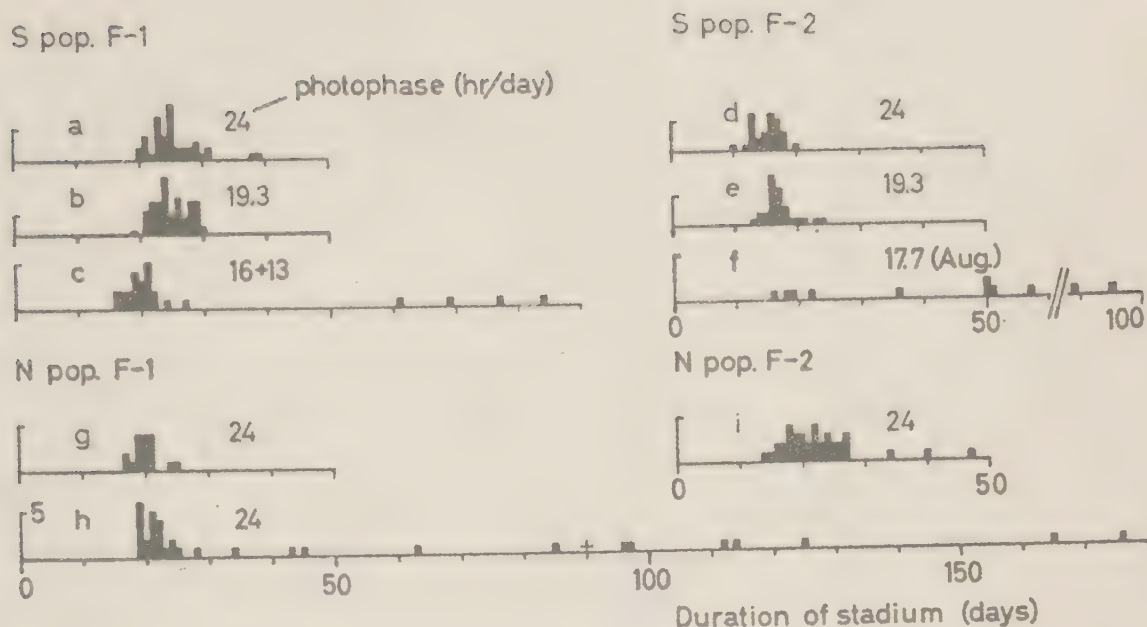


Fig. 5. Summary of the duration of the F-2 and F-1 stadia in all the experiments at long-day photoperiods and 20°C. For comparison, some results from other photoperiods are included for the S population (c, f). Photoperiod is shown as daylength (photophase) in hours. The bars on the ordinate indicate five specimens. A cross indicates the death of a larva. Only larvae that reached the respective instar between one week and five months after the start of the experiments are included.

- (c) The four diapausing specimens shown all belong to LD 13:11. Two specimens in a more intense diapause have been omitted for reason of space.
- (f) Limited to experiments begun near mid-August.
- (g) The larvae were in the F-2 instar when experiments began.
- (h) The larvae were in the F-5 (n= 1), F-4 (n= 12) and F-3 (n= 22) instars when experiments began.

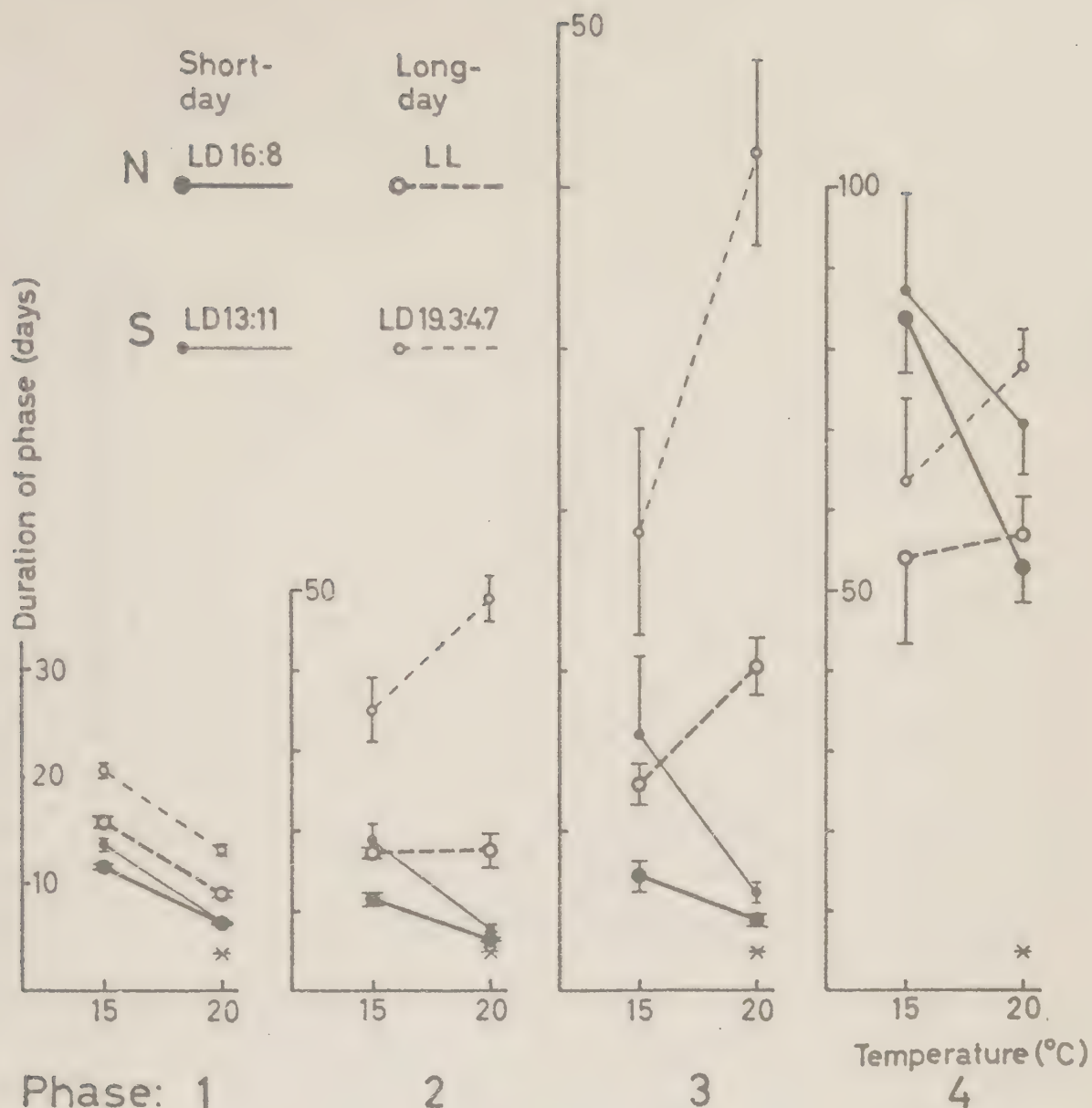


Fig. 6. Long-day and short-day responses in the first four phases of the F instar at 15° and 20°C. The mean and one standard error of the duration of each phase are shown. The ordinates are adjusted so that the duration of each phase for rapid (stimulated) development at 20°C is at approximately the same level (see asterisc). - N population: Experiments begun on 14 Jan. and on 21 Feb. 1975 at LL, 20°C, with larvae in the F-4 to F-2 instars. Transfer to the other experimental conditions took place when the ecdysis to the F instar was approaching. The number of larvae in each group was 8 or 9. - S population: Experiments begun on 30 Nov. 1973 at LD 19.3:4.7, 20°C, with larvae in the F-3 and F-2 instars. The design of the experiment was the same as for the N population. The number of larvae in each group varied between 6 and 9.

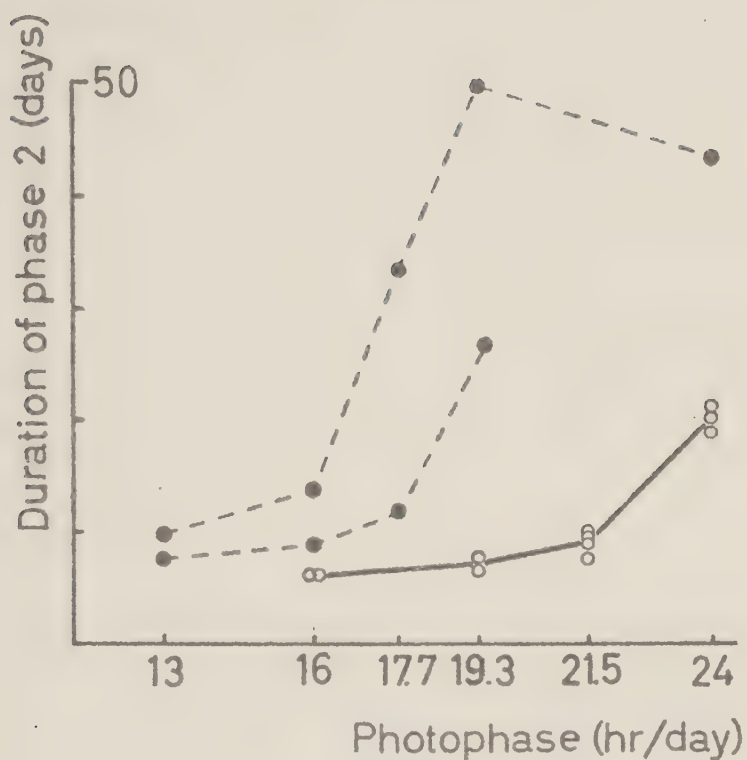


Fig. 7. Effect of different photoperiods on developmental time in phase 2 of the F instar. - N population (solid line): Experiment started on 17 Jan. 1974 in the F-1 instar. Each specimen is represented by a small circle. - S Population (broken lines): - Upper line: responses at constant photoperiods; - Lower line: a decrease in daylength preceded the entry into the F instar (from NORLING, 1976).

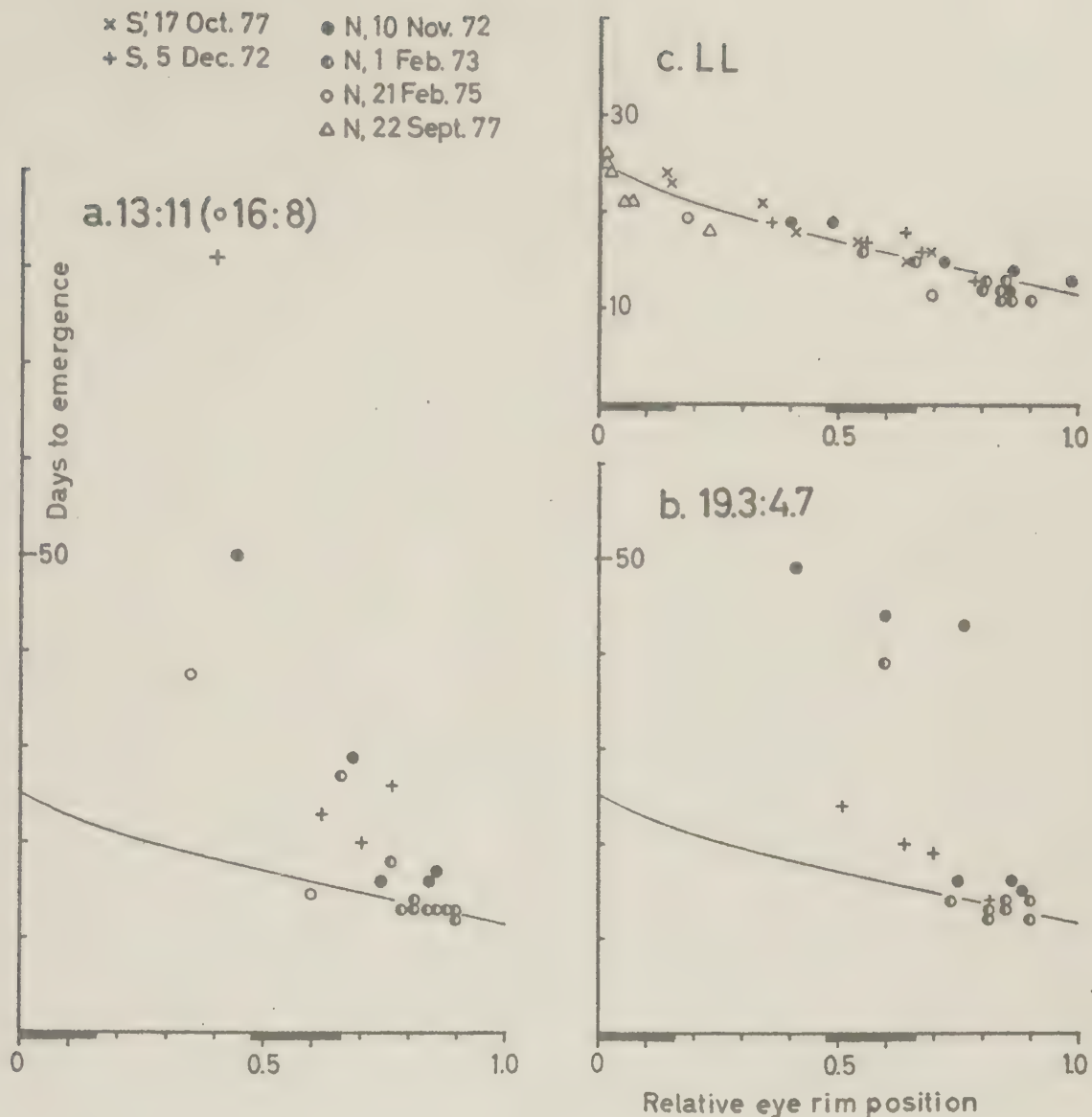


Fig. 8. Time to emergence for cold-treated F instar larvae in different phases of development at different photoperiods. Temperature 20°C. Origin of larvae (S', S, or N) and the date on which each experiment was begun are shown in the legend. The pretreatment of the larvae is shown in Table I. The abscissa illustrates the developmental stage (phase) of the larvae at the start of the experiments by the position of the posterior eye rim in its progression from the mesial tip of the larval eye (0) to a muscle scar behind it (1.0; cf. Fig. 2 in NORLING, 1976). Phases 1 and 3 are indicated by thickenings of the axis. The curve is a reference for rapid (stimulated) development, calculated from Fig. 2 in NORLING (1976).

THE LIFE CYCLE AND SEASONAL REGULATION OF COENACRION HASTULATUM
(CHARP.) IN TWO CLIMATICALLY DIFFERENT AREAS (ODONATA: ZYGOPTERA)

U. Norling

Abstract

The life-cycle of C. hastulatum was studied in southern (58°42'N) and northern (67°50'N) Sweden. In the southern area, the life-cycle duration was 1-2 years. The univoltine and semivoltine larvae separated as cohorts already before the first winter. During the second summer, the semivoltine cohort continued to develop slowly, while larvae that had overwintered in the last three instars, i.e. the univoltine cohort and older semivoltine larvae, rapidly proceeded to emergence, which mainly took place in June. Egg development was direct. In the northern area, the life-cycle duration was 3-4 years, and almost all larvae overwintered in the final instar before emergence. A marked frequency minimum in the penultimate instar during winter seemed to be produced during the late part of the third summer by a cohort separation suggestive of the one in the southern area. Experiments performed at different photoperiods partly explained the life-cycle pattern. Larvae overwintering below a certain, critical size, associated with the frequency minimum produced by the cohort separation, showed a slow development at long-day photoperiods, in particular in the penultimate instar. Larvae overwintering above the critical size rapidly proceeded to emergence under the same conditions. A 19.3 hr daylength produced a long-day response in the southern population only. The shorter days of September (southern population) produced a burst of moulting activity before a hibernation diapause, present in at least the three last instars, was induced. The critical overwintering size was concluded not to be a result of the previous cohort separation, which was assumed to be under separate environmental control. The early cohort separation may serve to reduce intraspecific competition and to add further refinement to seasonal regulation.

INTRODUCTION

Despite the great interest in dragonfly seasonality created by the early papers of CORBET (e.g. 1956, 1962), few studies on seasonal regulation in northern species and populations have been carried out (e.g. NORLING, 1971, 1976, 1981). In particular, the knowledge of populations living in subarctic, or high boreal areas is poor. In Sweden, Coenagrion hastulatum is one of the most successful damselfly species in the northern part of the boreal spruce forest area, and it is frequently the dominant coenagrionid species in small water-bodies all over the country.

The present paper is part of a survey of intraspecific variation in dragonfly larval growth and photoperiodic responses, carried out to illuminate how different species adapt their life-cycles to different conditions for development, both locally and between climatically different areas, i.e. southern and northern Sweden.

MATERIAL AND METHODS

Study sites

The principal study sites in southern Sweden were two barely separated, about 7m wide pools (A and B) in the large open Sphagnum bog Fjällmossen, 58°42'N, 16°31'E, 84m above mean sea level, about 20 km ENE Norrköping.

The vegetation, composed of Sphagnum, Utricularia, Eriophorum and low Carex, was mainly restricted to the edges. The bottom consisted of soft naked peat. Maximum depth in spring was approximately 1.5m in pool A and 0.7m in pool B. During the summer the level of both the water and the bog surface sank, and pool B could partly dry up. Pool A was studied during 1972 and 1973, pool B during 1972 only. Sampling was normally confined to the vegetation and took, if possible, place at 2-3 week intervals during the growth season. Temperature recording was performed in pool A during 1973 (see NORLING, 1976, Fig. 1).

In northern Sweden, three water-bodies in the Sappisaasi area, 67°50'N, 21°40'E, 15-22 km north of Vittangi and some 140 km north of the Arctic Circle, were examined. Except at site 3, sampling was restricted to three occasions during 1972.

Site 1.

About 25m wide pool in the large mixed-bog area Ripakaisenvuoma; 335m amsl. Sampling took place in floating Carex-Menyanthes mats and in other vegetation (mainly moss, Carex, Comarum, Menyanthes and Sparganium) down to 0.8 m depth. The invertebrate fauna appeared to be unusually rich for the area.

Site 2.

About 50m wide, possibly deep lake between the low peaks of the Sappisaasivaara mountain; 400m amsl. The lake is surrounded by boggy areas and a coarse Betula-Picea forest on the adjacent slopes. The sampling was performed at the edge in Menyanthes-Scorpidium mats with Carex and Eriophorum, and at the open water in large Carex and Potamogeton natans down to about 1m depth. The lake appeared to be partly spring-fed.

Site 3.

About 100m wide, very shallow lake at one end of a bog, situated in a flat area with coarse Betula-Picea forest; 375m amsl. Sampling took place in floating patches of low Carex and Eriophorum. The depth of the lake beside the patches was about 0.5m.

Daylengths and mean air temperatures in the two study areas are shown by NORLING (1981).

Collection methods and measurements

The bulk of the samples was obtained with a net (diameter about 0.3m) with 1x1mm mesh size. During 1972, the contents of this net were mostly placed on a plastic sheet for rapid examination, which was effective only for later instars (cf. NORLING, 1971, where this method was the only one used). To obtain samples also of the early, often extremely abundant instars, a small net (diameter 120mm) with 0.2x0.7mm mesh size, sufficient to retain all instars, was carefully used before collection with the large one. The contents of the small net were thoroughly washed out in wide plastic trays. Sometimes also part of the contents from the larger net was

searched in this manner.

During the 1973 study of pool A, the examination on a plastic sheet was largely replaced by washing in water through sieves with 20, 3 and 1mm mesh sizes, which permitted an easier separation of larvae and plant debris, and which increased the yield of smaller larvae. However, the small net, with which too much plant debris and peat could be avoided, still was the principal source of the earliest instars. This year particular care was taken to find the smallest larvae in each sample taken with the small net.

The collecting and searching methods produced a strong bias towards the later instars, but could still provide a good record of the presence of early instars. However, the time available at the sites was often a limiting factor for the detection of small larvae.

Larvae that could be identified in the field were usually also measured in the field and subsequently returned to their habitat (approximately, larvae with a head width above 1.7mm). Earlier instars were preserved in 80% alcohol for later examination. The head width of the three last instars were most easily and accurately measured in a wedge-shaped groove, as described by NORLING (1971). Smaller, live larvae, which could not be handled accurately enough for this method, were placed upon a soft, moist piece of cellulose sponge and measured with a scale lupe of 10x magnification. Preserved material was measured using an ocular grid in a dissection microscope. Repeated cross-checking showed a good accordance between the different methods, including the effects of preservation. Even in larger specimens the differences rarely reached 0.05mm.

In the later instars, the stage of development within the stadium was estimated by means of observations of eye development (cf. ELLER, 1963; NORLING, 1971). The larva was observed from the front with a lupe of 15-20x magnification, and the position of the pigmented anterior eye rim between the eye apex (0) and the base of the antenna (1.0) was estimated. The final (F) stadium was subdivided into seven phases, the last of which were defined by wing and labium development: 1: eye rim position (ERP) ≤ 0.62 ; - 2: $0.62 < \text{ERP} \leq 0.7$; - 3: $0.7 < \text{ERP} \leq 0.8$, the space between the black eye rim and the antenna turns reddish; 4: eye brownish or

black up to the antenna, costal rib still straight; - 5: costal rib corrugated, labium without visible signs of histolysis (15-20x magnification); - 6: histolysis and retraction of the tissues of the prementum; - 7: prementum empty, costal rib with dark spots.

The F-1 (final minus one, or penultimate) stadium to the F-3 stadium were subdivided into four phases: 1: $ERP < 0.58$; - 2: $0.58 \leq ERP < 0.66$; - 3: $0.66 \leq ERP \leq 0.7$; - 4: $ERP > 0.7$.

The lowest ERP value was usually 0.5, somewhat higher in the F instar, and the ERP value just before ecdysis (excepting the F instar) varied between little more than 0.7 up to 0.8.

Species separation

The shape of the prementum and the gill morphology were used for identification of the C. hastulatum larvae (cf. ANDER, 1926; PULKKINEN, 1925). Very young larvae are difficult, or impossible, to distinguish from those of many other coenagrionid species. However, at the southern study sites (Fjällmossen) Lestes sponsa (HANSEM.) was the only other regularly breeding zygopteran species, and during the study only two larvae could be identified as other coenagrionids. Therefore, all unidentified coenagrionid specimens, i.e. larvae with a head width below about 1mm, could safely be treated as C. hastulatum.

Other species were more troublesome in the northern area. C. armatum (CHARP.), not previously recorded from these latitudes, and concinnum (JOHANSS.) could not be distinguished from hastulatum up to a head width of about 1mm. Larvae of C. lunulatum (CHARP.), which are more similar to those of hastulatum, might even have been mistaken for the latter species up to a head width of 2mm. The size distribution of the identified non-hastulatum Coenagrion specimens (n= 50) does, however, not suggest that the results are seriously affected by regarding all young and unidentified larvae as hastulatum.

Experiments

Larvae for investigation of their photoperiodic responses were collected from the northern localities on 16-17 Sept. 1972, from the southern area on 12 Oct. 1972, 4, 17 and 31 Aug. 1973, 26+29 Oct. 1973, and 2 May 1974. The material from Fjällmossen was often supplemented with material from other sites in the southern area.

The 4 Aug. to 31 Aug. collections were kept at approximately natural temperatures and photoperiod (incl. Civil Twilight) until the start of the experiments; all other material was immediately stored at 0-4°C and occasionally interrupted darkness (see also NORLING, 1981). Acclimation to experimental temperatures was allowed to take 5 hours or more. Experiments on both populations were performed at 20°C and LD 13:11 (13hr light: 11 hr dark), 19.3:4.7 and LL (continuous light). The southern population was also examined at LD 16:8, 20°C and LD 13:11 and 19.3:4.7 at 15°C. The procedure and equipment have been described elsewhere (NORLING, 1976, 1981).

Diapause

Diapause is here used in a wide sense: a prolongation of development, or reduction in growth rate, that is not directly caused by adverse conditions (cf. BECK, 1980). Diapause usually serves the preparation for adverse conditions, e.g. winter, but can also regulate development for other purposes. In Odonata, there is often a continuous transition from a slight prolongation of development to an almost total arrest, that lasts for months (e.g. NORLING, 1971). In this paper, an ecdysis interval approximately twice that at the maximum rate of development is arbitrarily chosen as a limit for the use of the term. At 20°C, this limit is about 30 days for the F instar and 15-20 days for earlier instars.

RESULTS

The life-cycle of the southern population

Emergence started during the last days of May and probably continued to the end of June (Fig. 1, upper graphs). After oviposition, which mainly took place in June and early July, egg development was direct, and larvae of the next generation started to appear in the July samples. During 1973, the best documented year (Fig. 1c), hatching probably started during the very last days of June and continued at least throughout July. The hatching peak appeared to be situated in the early part of the period.

During subsequent development, the larvae gradually separated into two cohorts with different growth rates, as previously demonstrated for *C. puella* (L.) in Britain (PARR, 1971; LAWTON, 1972). In the present material the process of separation was revealed in greater detail than before. The fast-growing group appeared to hatch earlier than the slow-growing one, and the separation was produced by the combined effect of a difference in the size increase per ecdysis and a relatively slight difference in ecdysis intervals (Fig. 1c). The difference in growth rate was accentuated by decreasing temperatures from the start of hatching (cf. the temperature graph in NORLING, 1976, Fig. 1).

The last ecdyses for the season took place near the shift September-October. The fast-growing group spent the winter in the F-2 (9th) and F-1 (10th) instars, in 1973 most likely also in the F (11th) instar. The larvae in the slow-growing group did not usually develop further than the 8th instar before winter. These 8th instars had the size of a 7th instar of the fast-growing group.

After overwintering, ecdyses started about mid-May. Larvae that had spent the winter in the last three, possibly four instars, which includes the whole fast-growing group, showed a most rapid development, and they emerged soon. The fast-growing group is thus univoltine, and it finishes larval development after 11 instars. The slow-growing group continued development at a reduced rate, which was particularly evident in the F-1 instar. The F instar was reached during September, and development halted in an early

part of the stadium (diapause). After the second overwintering, the remaining development was rapid, and these semivoltine specimens were among the first to emerge during the season. The total number of instars required to complete development was predominantly 13.

The development in pool A during 1972 was similar to that during 1973 (Fig. 1a). However, in pool B, the proportion of fast-growing, univoltine larvae, hatching during 1972, was smaller than in pool A, and the slow-growing, semivoltine group grew even slower than in pool A. In this year, most of the Carex-Eriophorum patches, the preferred habitat for C. hastulatum, dried up in pool B during July. This forced many larvae out into Sphagnum moss and, probably, naked peat, which can be assumed to be less suitable habitats. A decline in growth rate was also noted among the semivoltine larvae from the preceding year.

Some material of C. hastulatum from other localities suggested that different proportions of one and two year development, as described above, is a normal feature in southern Sweden. In the southernmost part, univoltinism seemed to dominate, and the winter was mostly spent in the F-1 instar. A two year development appeared to dominate at a site some 30km W Fjällmossen, and here a massive overwintering in the F instar took place.

Life-cycle of the northern population

The upper graphs in Fig. 2 suggest that emergence during 1972 started at about the summer solstice at site 1 and 3, but later at site 2, which can be supposed to warm up slower during the spring. Furthermore, nearly all specimens must have emerged in an early, well synchronized peak.

At all sites reproduction was successful during 1972, when the weather was unusually hot during late June and the first half of July, i.e. the period of maturation, reproduction and egg development. The new generation, the 1972 year-class, had appeared already on 21-22 July (Fig. 2). The development during the first summer was more synchronous than in the southern population. The size of the northern 1972 year class in the samples from September, when growth had halted, suggested that the conditions for growth were

most favourable at the shallow site 3, and least favourable at site 2, which presumably was spring-fed.

In the June samples, the preceding (1971) year-class was present only from site 1. The small size of these presumably overwintered larvae reflects a relatively cool summer during 1971. At site 3, the 1971 year-class showed up later, but it was less conspicuous than at site 1. At the coal site 2, reproduction appeared to have failed completely during 1971. During the summer of 1972, the 1971 year-class generally reached a head width somewhere around 2mm. Precisely what happened with larvae that were at, or just below this size at the beginning of 1972 was not readily apparent, and it probably varied. It appeared that they either reached the F instar, or a stage with a head width about 2.5mm (cf. below).

The winter before emergence was almost exclusively spent in the F instar, the size of which was distinctly greater than in the southern population. The data from site 1 suggest that also the few larvae that had overwintered in the F-1 instar emerged during the summer, but later than the main group. At the time of the June sample, two such larvae were in the process of entering the F instar. Larvae in the preceding instars were not yet ready for ecdysis at this time, and on 21 July they were accumulating in the F-1 instar. This pattern of accumulation was similar at all three sites, and the entry into the F instar probably started at the end of July, at least at site 1 and 3. On the average, the F instars spent the winter in a slightly more advanced state than in the southern population.

The remarkably low frequency of overwintering larvae in the F-1 instar appeared to arise from a secondary cohort splitting during late summer, because no corresponding frequency minimum was present in the June or July samples.

These results suggest a dominating 3 year life-cycle at site 3, which appears to be corroborated by the July 1974 sample. At site 1 and 2, the life-cycle duration apparently was 3-4 or 4 years. The size distribution of the June sample from site 1 is distinctly quadrimodal, and the September sample from site 2 would probably have been, if the missing 1971 year-class had been present. The life-cycle duration certainly varies between different year-classes.

Photoperiodic responses of the southern population

When larvae, collected during the cold season and stored at low temperatures, were subjected to LD 19.3:4.7 or LL at 20°C, the development was initially similar to that observed during the field studies (Figs. 3, 4; Tables I, II). Larvae overwintering in the last three instars with few exceptions proceeded rapidly to emergence, while larvae in the young semivoltine group showed a greatly delayed development. The prolongation of development appeared to occur in all instars present during the experiments, but was most distinct in the F-1 instar (Table I). In other words, the size separation between the semivoltine and univoltine cohorts during their first winter also separated two different types of response to these long-day photoperiods.

Compared to the above photoperiods, LD 16:8 slightly, or moderately, prolonged the development of the larvae in the last three (overwintering) instar groups. This effect was particularly noticeable in the overwintering F-1 instars (Fig. 4). On the other hand, the development of the young semivoltine larvae was distinctly more rapid than at the long-day photoperiods, and it even appeared to be close to the physiological maximum rate in some specimens.

Also at LD 13:11, many specimens in the last three instar groups still showed an insignificant, or moderate, prolongation of development, but a stronger delay could occur, as in the 5 Dec. 1972 experiment (Fig. 4) and in overwintering F instars (Table II). The rate of development in the semivoltine group was not as greatly retarded as at long-day photoperiods, but it was slower than in most larvae at LD 16:8. A really distinct, short-day induced hibernation diapause was mostly absent in these experiments, but weak diapause phenomena were present in all examined instars at LD 13:11 (Table I). A comparison between the 5 Dec. 1972 and 7 May 1974 experiments reveals seasonal differences in the intensity of the responses.

Occasionally, larvae in the F-2 instar group could, like the semivoltine group, enter slow development at long-day photoperiods. On these occasions, a supplementary instar was added to the development. This long-day diapause did not take place at LD 19.3:4.7, 20°C (n = 9; only the F stadium was prolonged in one larva), but

at LL, 20°C (one of four) or at LD 19.3:4.7, 15°C (one of five). In an experiment at LD 19.5:4.5 and slowly rising temperatures (8°C on 27 March to 20°C on 12 June, 1971), designed for another species, two "F-2" instar specimens of C. hastulatum from the vicinity of Lund (55°40'N) were present. Both reacted with a strong diapause and two supplementary instars.

These experiments on "overwintering" larvae suggest that the different rates of development in the univoltine and semivoltine cohorts after the first winter are not predetermined. As in Aeshna viridis EVERSM. (NORLING, 1971), the critical overwintering size, which separates the two rates of summer development in a population, is not absolutely fixed: Extreme long-day photoperiods and low temperatures probably increase the critical size into the range of the univoltine cohort, and an "intermediate" photoperiod, as LD 16:8, allows a relatively rapid development also in the semivoltine cohort.

The temperature-induced difference in the rates of rapid development observed between 15°C and 20°C (Table II) was always near a factor of two, as in Aeshna viridis and Leucorrhinia dubia (VAN DER LIND.) (NORLING, 1971, 1976, 1981), which suggests a lower temperature threshold for development near 10°C in the examined instars.

The temporal separation of emergence between larvae overwintering in the last three instars seemed, at long-day photoperiods, to be simply related to the normal duration of the involved stadia at rapid development. The small variation in ocular development in the F instar groups at the beginning of the experiments (cf. Fig. 1) was, however, not found to be well correlated with the variation in the time to emergence.

The experiments initiated during August and early September (20°C only) demonstrated photoperiodic responses, which were appropriate for this time of the year. Already in the first, and largest of these experiments, begun on 6 Aug. 1973, the responses to LD 13:11 closely resembled what happened in the field later on. The larvae in the F-1 instar (old semivoltine) entered the F instar synchronously after 21-26 days (n=14), whereafter a strong diapause was induced. The F stadium duration was 102.3 ± 15.9 days ($\bar{X} \pm SD$; range 72-122; n=8). If the larvae were transferred to LD 19.3:4.7 in close connection with the ecdysis, the diapause

was soon broken and the F stadium duration was 23-27 days ($n = 7$). If the transfer took place 5-12 days before the ecdysis, the diapause was averted and the F stadium duration was only 17-20 days ($n = 5$).

Larvae that were in earlier instars on 6 August (two F-4 and two F-3 from the univoltine cohort, nine F-2 from the old semivoltine cohort) moulted within 16 days. Specimens moulting within 14 days also moulted a second time before entering diapause, which took place 21-29 days after 6 August ($n = 12$). The moulting interval was particularly short (12-16 days, $n = 6$) in larvae, which performed their first ecdysis 7-14 days after the start of the experiment, when probably no lingering long-day effects were present. Including Civil Twilight, the daylength on 6 August is about 18 hours in the southern area (cf. NORLING, 1976). The exact stadium duration of the diapausing F-2 ($n = 2$) and F-1 ($n = 3$) larvae was not recorded, but it exceeded 50 days. For the youngest larvae it appeared that the premature short-day treatment produced a hibernation diapause in an earlier instar than would have occurred in the field.

Overall, the last ecdysis before diapause at LD 13:11 was remarkably well synchronized, and took place after 21-26 days ($n = 25$), except in two larvae, which entered the diapause instar after 16 and 29 days, respectively. In a smaller experiment, started on 19 August, the results fitted entirely into the above pattern both at LD 13:11 (four F-2, three F-1) and LD 16:8 (two F-2, one F-1).

In the 6 August experiment, the responses to LD 19.3:4.7 were as follows. Of seven larvae in the F-1 instar, two entered the F instar after 18 days, and emerged after a further 15 days. The other five postponed their entry into the F instar until after 31-41 days, and thus displayed a long-day diapause. The duration of the F stadium in these larvae was almost the same as that for long-day diapausing larvae in the 7 May 1974 experiment (Table I): 31.3 ± 4.4 (27-37) days ($n = 4$). Evidently a strong long-day diapause is absent in the F instar.

Larvae in earlier instars (one "F-4", one "F-3", two F-2) moulted after 7-16 days and thereafter displayed a

long-day diapause. The two youngest larvae also made one supplementary moult each. The LD 19.3:4.7 group from 19 August (four F-2, three F-1) did not differ significantly from this pattern.

In the 2 September experiment all larvae at LD 13:11 (four F-2, seven F-1) moulted within 2-11 days and then entered the hibernation diapause. At LD 16:8 (three F-2, six F-1) the larvae moulted within 1-14 days, but two of the F-2 larvae moulted once more before entering diapause. At LD 19.3:4.7 (four F-2, six F-1) ecdysis took place within 1-10 days, but all larvae except one (F-2) now showed rapid development as in the winter experiments.

The August-September experiments show that LD 13:11 induces a distinct hibernation diapause in at least the three last instars at this time of the year. Before this occurs, any previously induced long-day diapause is terminated, and a delay in the onset of the hibernation diapause can permit some rapid development. The hibernation diapause can be terminated by long-day photoperiods. Also LD 16:8 shows these short-day effects at this time, but it probably permits a longer period of rapid development before the onset of the hibernation diapause. In late instar larvae, LD 19.3:4.7 generally produces an immediate diapause response in early August, but hardly a month later the response has changed almost completely to a stimulation of development.

Photoperiodic responses of the northern population

Only two experiments were performed with this population, both during the winter 1972-73 (Fig. 5; Table III). At LL, 20°C, which can be considered as roughly normal summer conditions in the northern study area (NORLING, 1981), the critical overwintering size for rapid development was similar to that observed in the field, i.e. just below the F-1 instar, or below a head width of 2.9mm. Larvae below this size at the beginning of the experiments developed slowly at LL, and even the largest of them (head width about 2.6mm) developed as F-3 instars despite their size. Also in this population the F-1 instar was responsible for a

considerable part of the prolongation of development, but the intensity of the long-day diapauses were generally less than in the southern population, except perhaps in the F instar.

LD 19.3:4.7 did not act as a long-day photoperiod in these experiments. As far as can be seen in the few specimens tested, the development of the F-1 instar group was slowed down significantly. Larvae smaller than this developed more rapidly at LD 19.3:4.7, and partly at LD 13:11, than at LL. To some extent, the effects of LD 19.3:4.7 on the northern population can be compared with that of LD 16:8 on the southern population.

Diapause-like phenomena were recorded in all examined pre-final instars at all tested photoperiods. However, the responses of the F instar were somewhat intriguing. No diapause ever occurred at LD 13:11 when the F instar was reached at this photoperiod. At LD 19.3:4.7 five out of the seven larvae in the F-3 instar group that reached the F instar within 50 days showed no diapause, whereas the remainder of these larvae showed, as far as could be controlled, a fairly intense diapause. At LL an F instar diapause appeared to be present in at least two larvae.

In overwintering F instars, there was an increasing delay of the emergence with shorter daylengths, as in the southern population, and at LD 13:11 in the 10 Nov. 1972 experiment the response was in the range of diapause as defined here. As in the southern population, the range in ocular development at the start of the experiments (cf. Fig. 2) showed only a slight correlation with the observed variation in the time to emergence.

In the latest of the two experiments, a more rapid development than in the earlier one occurred in overwintering F instar larvae and in the earliest of the examined instars, suggesting the usual promoting effect of exposure to low temperatures. However, the total durations of the F-2 to F stadia in the experiments appeared not to show these seasonal differences.

DISCUSSION

The backbone of the seasonal regulation of C. hastulatum is provided by the same two-step photoperiodic reaction as in many other odonate species: Autumn emergence is prevented by a long-day induced prolongation of development during late summer. After exposure to the short days and low temperatures of the autumn and winter, which also suppress development, the long days of the spring promote development, and emergence can take place (see CORBET, 1980, for references).

A surprisingly good simulation of the natural summer development was achieved when "overwintering" larvae of C. hastulatum were suddenly subjected to constant artificial summer conditions, i.e. favourable temperatures and long-day photoperiods, as previously demonstrated also for Aeshna viridis and Leucorrhinia dubia (NORLING, 1971, 1976, 1981). Individuals, which had overwintered above a certain genetically and, to some extent, environmentally determined critical size, were stimulated to rapid development by the long days, and they soon emerged. At this time, rapid development was further enhanced by the previous exposure to low temperatures, and even short-day photoperiods often did not more than slightly delay emergence. Larvae, which had overwintered below the critical size, showed the long-day induced prolongation of development, which culminated in the F-1 instar.

The late summer experiments with the southern population explained the course of events during August-September. The long-day diapause in the F-1 instar is terminated by the shortening days, the F instar is reached synchronously, and a short-day induced hibernation diapause virtually halts further development. Less advanced larvae, in particular those of the younger, univoltine cohort, show some relatively rapid development during the period of intermediate and short days immediately preceding the initiation of the hibernation diapause. The univoltine cohort seems to escape a noticeable long-day diapause because the most sensitive instars are not reached until very late in the summer. The sensitivity to short days and the intensity of the short-day diapause in the early autumn experiments may be governed by the long previous exposure to long days and favourable temperatures.

The critical overwintering size is a key factor in the seasonal regulation of a population, because it ultimately determines the least advanced stage in which the winter before emergence can be spent. The exact emergence period is largely determined by the range of developmental stages above the critical size that is present during this winter, and the time it takes to reach emergence from each of these stages under natural conditions, i.e. the rate of development. When the last winter is spent in several instars, as in the southern population of C. hastulatum, an increased synchronization of emergence can be achieved by a system of rising lower temperature thresholds for development in the different instars (CORBET, 1957; LUTZ, 1968) or by different diapause characteristics of these instars (ELLER, 1963). However, no such mechanisms were detected in C. hastulatum (cf. Fig. 1). An early and relatively well synchronized emergence appeared to be achieved principally by means of a high rate of development.

The responses at LD 16:8, 20°C (Fig. 4) might suggest that some synchronization occurs between larvae that overwinter in the F-2 and F-1 instars. It is, however, doubtful if these responses can contribute to synchronization in the field. LD 16:8 is the natural photoperiod at mid-April (Civil Twilight added to the photophase), a time when it is too cold for any development to occur (cf. NORLING, 1976, Fig. 1). Moreover, larvae overwintering in the F instar are not included in the synchronization. SAWCHYN (1972) reported similar experimental results with larvae of C. angulatum WALKER at LD 12:12, 16.5°C.

Most of the differences in development and photoperiodic responses found between the two populations of C. hastulatum can directly be related to climatic differences between the two study areas (see NORLING, 1981, for more detailed data on climate). The difference in voltinism is a natural consequence of the much shorter and, on the average, cooler summers in the northern area, and the extremely long daylengths necessary for a long-day response in the northern population are related to the extreme photoperiodic regime which this population is adapted to. In the northern area, there is continuous light during most of the summer, and LD 19.3:4.7, which does not act as a long-day photoperiod

in the northern population, bears the same relationship to air temperature during late summer - autumn as LD 14:10 in the southern area.

The short, cool summers of the north also restrict the time available for maturation, reproduction, egg development and the very first part of larval development (the prolarva, possibly the second instar), which probably must be completed before winter conditions can be survived. The end of the emergence period in the southern population does not appear to be determined solely by climatical factors, but that in the northern population probably is. An extremely early emergence is needed in order to allow the above sequence to be completed before winter (cf. the more detailed discussion in NORLING, 1981). Properties of the northern population related to this requirement are the larger critical overwintering size and the slightly more advanced state of development of the overwintering F instars.

Also the relatively weak long-day diapause in the northern population is connected with the short summers. A lower diapause intensity is sufficient to prevent autumn emergence in the northern area, and the larvae must not be prevented to reach an advanced stage before winter, if the emergence is to take place early during the following summer. Similar population differences between these two areas have also been reported in Leucorrhinia dubia (NORLING, 1981).

One of the most intriguing aspects of the life-cycle of C. hastulatum is the early splitting of the recently hatched larvae in the southern population into two cohorts with different growth rates. PARR (1970) and LAWTON (1972) showed this phenomenon in C. puella, and own preliminary observations indicate that also C. pulchellum (VAN DER LIND.) in southern Sweden is similar to hastulatum in this respect.

The difference in growth rate between the cohorts during the second summer can be entirely explained by the position of the critical overwintering size in the gap between the cohorts, and it is not a predetermined continuation of the developmental rates from the previous year (p. 11). The only experiment related to the development during the first summer shows that

the univoltine cohort displays the same kind of responses to photoperiod during late summer as the older, semivoltine group of larvae (pp. 12, 13). However, there are no experimental results related to the separation of the cohorts during the first summer.

The remarkably distinct difference between the cohorts as concerns the size increase per ecdysis appears to be too large and abrupt to be explained entirely by a continuous variation like the one producing different numbers of instars in Aeshna cyanea (MÜLL.) (SCHALLER, 1960). Because the ratio between the number of specimens in each cohort appeared to be changed by environmental factors affecting growth, the cohort separation is probably not genetically predetermined. Although both genetical variation, and environmental factors acting during the egg stage (cf. SCHALLER & MOUZE, 1970), certainly contribute to this variation in development, the final cohort determination can tentatively be assumed to be the result of environmental factors acting in the larval stage, producing responses, which anticipate and refine the more definite cohort separation that is tied to the critical overwintering size during the spring.

Late hatching specimens encounter a somewhat different environment as regards temperature and photoperiod than the early hatching ones. Specific responses to these factors are particularly likely to affect the final stages of the cohort separation and the exact location of the winter frequency gap. At high densities intraspecific competition in the hatching population may be important during the early part of the process. LAWTON et al., (1980) showed that food shortage could both increase the ecdysis intervals and reduce the size increase per ecdysis in larvae of Ischnura elegans (VAN DER LIND.). If late hatching specimens of C. hastulatum are affected in this manner by competition from somewhat older larvae, the variation in rates of development and number of instars would be enhanced and also become related to food availability.

An early cohort separation has the advantage to reduce competition between larvae that still would be separated later at the critical overwintering size. The semivoltine cohort can then perform more of its growth than otherwise during a time when univoltine larvae are either absent or very different in size.

In the northern area there are indications of a somewhat similar cohort splitting during the third summer, which bears the same temporal relationship to emergence as the first summer in the southern population. The peak of overwintering larvae with a head width around 2.5 mm (the F-3 and F-2 instars) may have been produced by a late accumulation in this size while most F-1 larvae entered the F instar. The frequency minimum thus produced is associated with the critical size, as the one in the southern population. The position of the frequency minimum in the northern population just above the critical size has an effect on phenology comparable with that of an increase in critical size. A higher true critical overwintering size (i.e. one above the F-1 instar) may be desirable in the population, but it may be impracticable in this species if the long-day diapause in the F instar is not intense enough to prevent attempts at autumn emergence when this instar is reached during the early part of the season.

A cohort splitting of a similar type, producing a low frequency of overwintering larvae near the critical size, and adding further refinement to seasonal regulation, is apparently present also in Leucorrhinia dubia (NORLING, 1981). In this slow-growing early species the frequency minimum is partly, or completely situated in the early part of the F stadium, and the critical overwintering size is just above, or slightly "within", the F-1 instar. Differences in the responses to late summer - early autumn daylengths occurred in different developmental stages so as to produce the frequency minimum before winter. It is possible that this kind of cohort splitting is a widespread phenomenon in the seasonal regulation of phenologically early species with an average life-cycle duration exceeding one year.

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Table I. Southern population. Total duration in days of different larval stadia in experiments on overwintering small (semi-voltine) larvae at 20°C and different photoperiods. Durations of stadia earlier than F-2 (head width always <2.5mm) are pooled. Mean $\pm$ standard deviation, range (in parenthesis) and number of values are shown, for the pooled data of earlier stadia also the number of larvae. Divergent single values and some minimum values are shown separately in parentheses.

	Date of experiment	LD 13:11	LD 16:8	LD 19.3:4.7	LL
<F-2, <2.5mm	5 Dec. 72	16.2 $\pm$ 3.7 (9-25)	18/9	32.3 $\pm$ 8.8 (14-46)	15/5 25.1 $\pm$ 5.9 (17-41) 16/6
	30 Nov. 73	--	8.7 $\pm$ 0.6 (8-9)	3/2	--
	7 May 74	13.2 $\pm$ 3.6 (10-22)	10/4	18.0 $\pm$ 2.9 (14-23)	13/5 --
F-2	5. Dec. 72	24.0 $\pm$ 5.5 (18-33)	7	25.3 $\pm$ 4.6 (20-30)	4 32.8 $\pm$ 3.7 (28-37) 4
	30 Nov. 73	--	8.0 $\pm$ 1.4 (7-9)	2	--
	7 May 74	16.0 $\pm$ 2.0 (14-18)	3	20.3 $\pm$ 3.2 (18-25)	4 --
F-1	5. Dec. 72	42.5 $\pm$ 17.5 (25-64) ($>47- >54$)	4 2)	($>45- >63$)	4) 43 ($>42- >59$) 1 2)
	30. Nov. 73	--	9.5 $\pm$ 0.7 (9-10)	2	--
	7 May 74	23.7 $\pm$ 12.5 (11-36)	3	60.3 $\pm$ 17.7 (39-80)	4 --
F	5. Dec. 72	32 (>22)	1 1)	--	--
	30 Nov. 73	--	15.0 $\pm$ 0.0 (15)	2	--
	7 May 74	25.3 $\pm$ 7.1 (19-33) (80)	3 1)	29.0 $\pm$ 4.8 (26-36)	4 --

Table II. Southern population. Effect of temperature and photoperiod on larvae overwintering in the last three instars: total duration of the F-1 and F' stadia, and time to emergence for larvae overwintering in the F instar (days). Experiment started on 30 Nov. 1973. Mean, standard deviation, and number of values are shown as in Table I. An asterisc indicates values for diapausing larvae exhibiting supplementary moulting. See also note under Fig. 4.

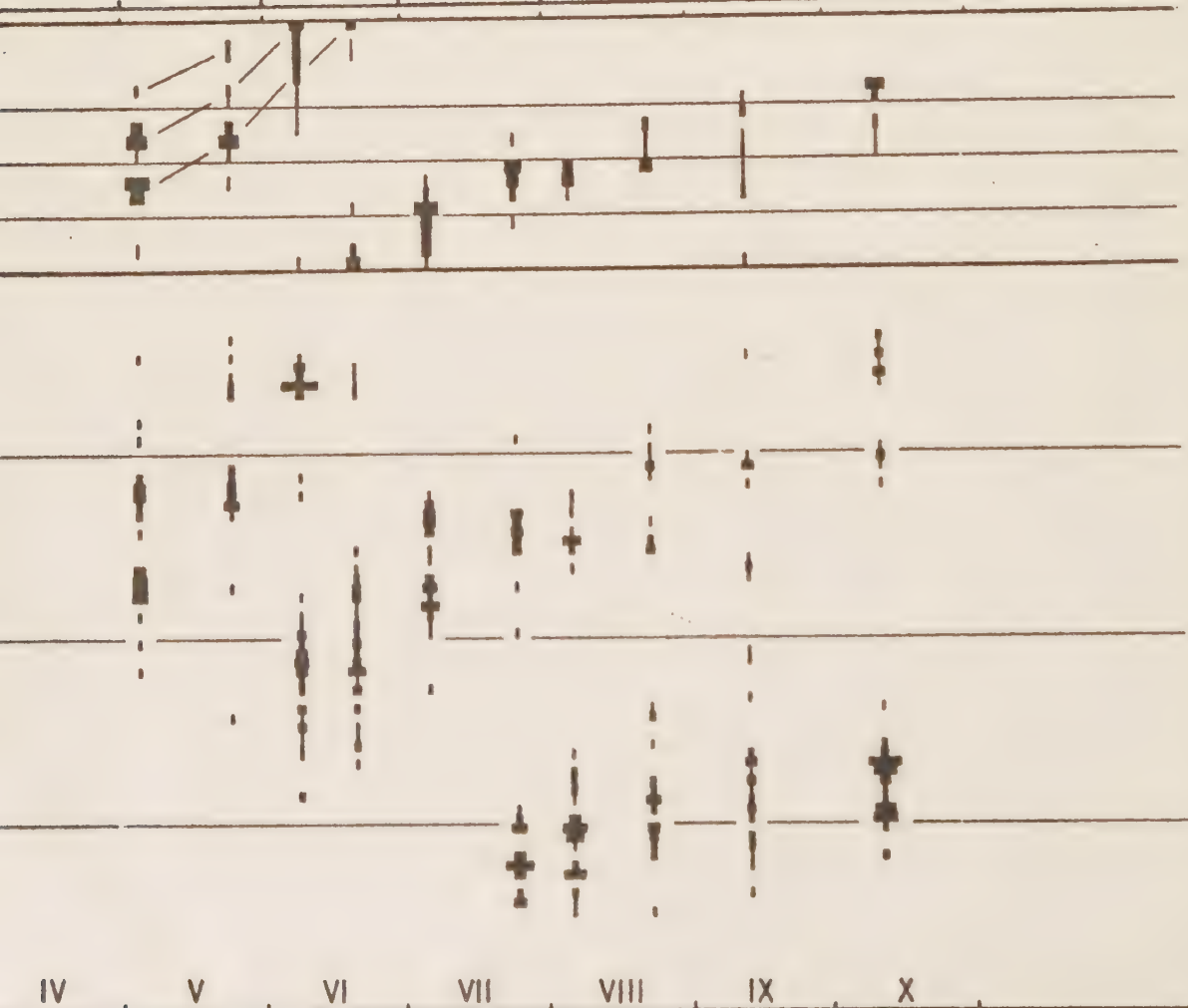
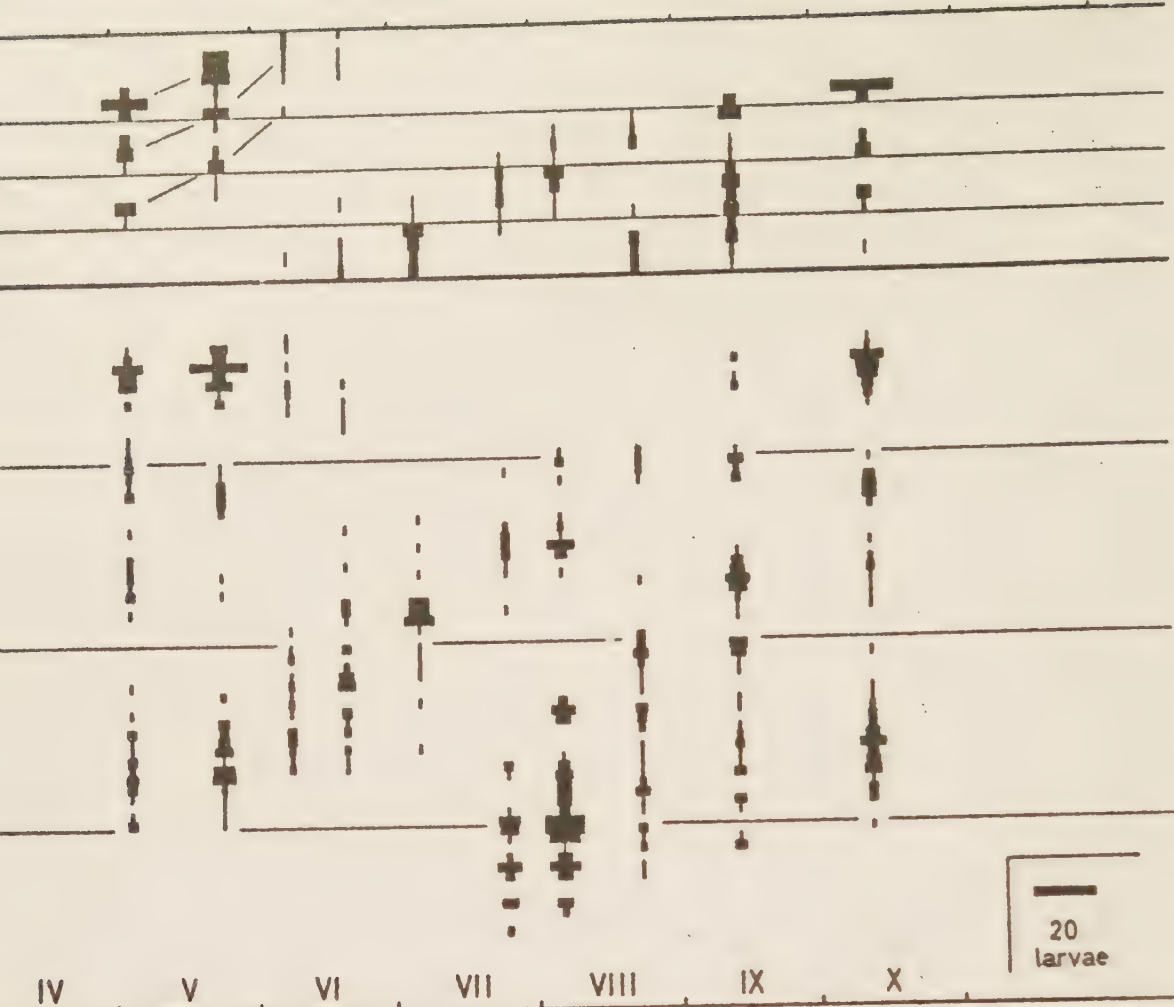
		LD 13:11	LD 16:8	LD 19.3:4.7
20°C	F-1	--	12.0 ± 0.8 (11-13) (19*)	8.5 ± 1.3 (7-10) 4
	F	19.7 ± 1.5 (18-21) 3	17.1 ± 1.7 (15-21) (82*)	14.6 ± 0.7 (13-15) 11
	To emergence from F	32.0 ± 12.1 (21-45) 3	20.4 ± 3.0 (17-25) 5	17.0 ± 0.0 (17) 4
15°C	F-1	--	--	16.8 ± 1.0 (16-18) (67*) 4 1)
	F	32.7 ± 1.5 (31-34) (80) 1)	--	27.8 ± 1.8 (25-32) 11 (39; 50*) 2)
	To emergence from F	49.5 ± 14.7 (35-68) 4	--	34.0 ± 0.8 (33-35) 4

Table III. Northern population. Total duration of different larval stadia in experiments on larvae overwintering in instars earlier than F-1, and time to emergence for larvae overwintering in the F instar (days). Temperature 20°C. Mean, standard deviation, and number of values/larvae are shown as in Table I.

	Date of experiment	LD 13:11	LD 19.3:4.7	LL
<F-2, < 2.5mm	10 Nov. 72	28.8 ± 7.2 (17-39) 9/6	16.5 ± 3.8 (11-23) 8/6	22.3 ± 7.6 (11-32) 8/5
	31 Jan. 73	16.3 ± 5.9 (10-26) 6/4 (46) 1/1)	13.3 ± 5.6 (9-24) 8/5	16 1
F-2	10 Nov. 72	13.0 ± 2.4 (10-18) 15	14.6 ± 4.0 (9-23) 18	17.8 ± 4.0 (12-26) 9
	31 Jan. 73		(65) 1)	
F-1	10 Nov. 72	19.6 ± 6.4 (9-30) 16	17.4 ± 5.2 (8-31) 17	36.8 ± 6.7 (28-48) 8
	31 Jan. 73			
F	10 Nov. 72	18.7 ± 2.2 (16-23) 12	17.4 ± 0.9 (16-18) 1)	38.0 ± 17.0 (26-50) 2
	31 Jan. 73		73.0 ± 13.0 (60-86) 3	
To emergence from F	10 Nov. 72	35.2 ± 16.8 (21-61) 6	19.4 ± 3.6 (15-25) 5	17.2 ± 0.8 16-18 5
	31 Jan. 73	16.8 ± 1.7 (14-19) 10	15.5 ± 1.4 (14-18) 10	
		(55) 1)	(28) 1)	13.9 ± 0.7 (13-15) 7
				(21) 1)

1) The F instar was reached within 50 days from the start of the experiments.

2) Observations at the end of the experiments show that an additional 8 larvae were in diapause.



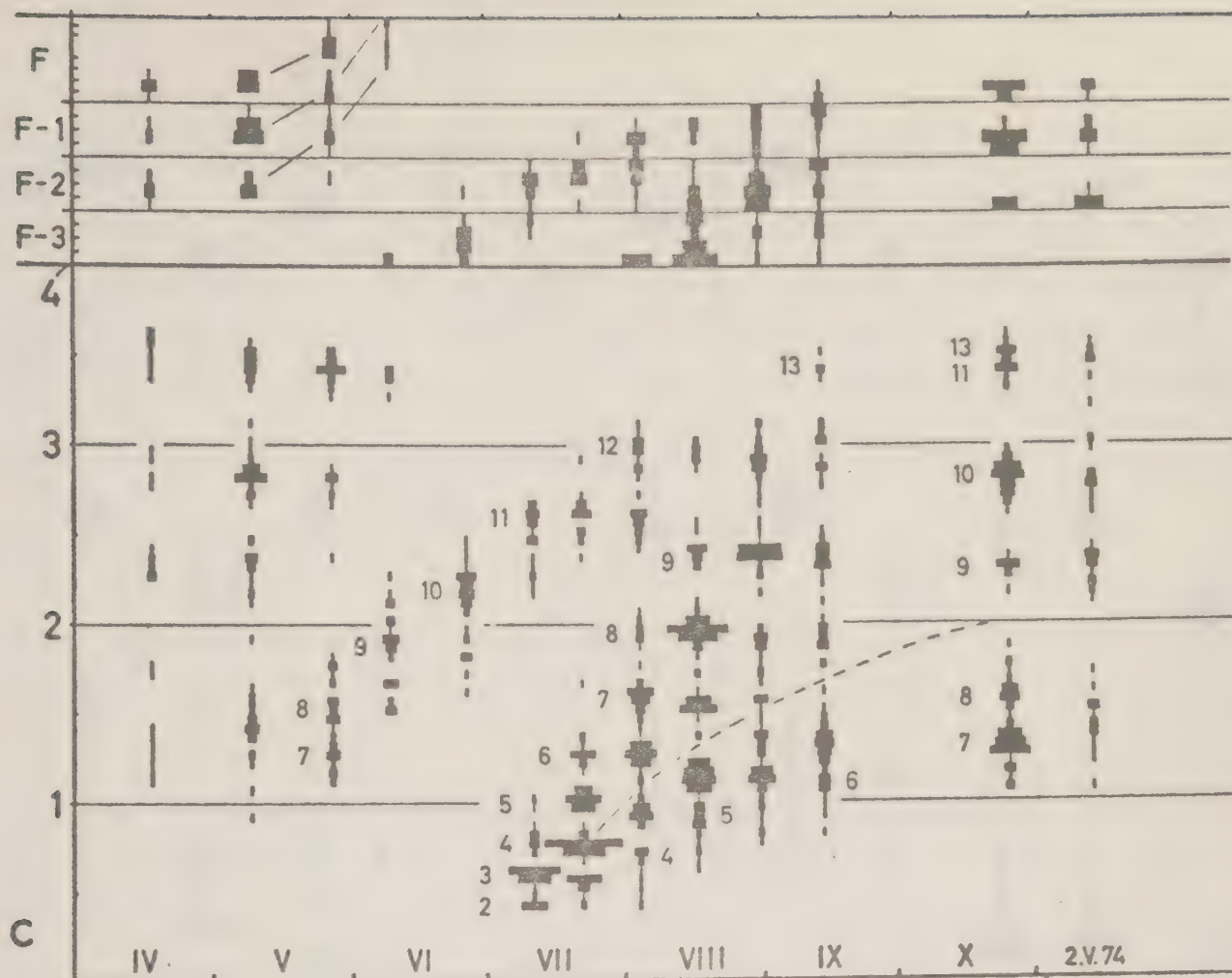


Fig. 1. Larval development of *C. hastulatum* at the study sites in southern Sweden, $58^{\circ}43'N$. The months from April to October are shown with roman numerals. - (a) Pool A, 1972. - (b) Pool B, 1972. - (c) Pool A, 1973. - Lower part: Kite diagrams showing head width frequency distributions in different samples. The size-classes are 0.05 mm. In (c) the frequency peaks, which represents different instars, are assigned instar numbers, with the prolarval instar counted as the first one. The incipient separation of the univoltine and semivoltine cohorts in the 1973 year-class is approximately indicated by a broken line. For reasons of space, this year-class is shown in a scale reduced to 50% in the 11 July to 4 August samples. - Upper part: Graphic presentation of the development in the last four instars, showing the distribution of larvae among the arbitrary instar subunits (phases) on the different dates. The ordinate shows the approximate relative duration of each stadium, and each phase in the F instar, at $20^{\circ}C$ and rapid development. In the F-3 to F-1 instars the phases are shown equal, but their true relative duration is uncertain and varies between specimens. Each larva is represented by the same area as in the lower part, except when the scale is reduced there. A few larvae, where the instar assignment is most uncertain, have been omitted.

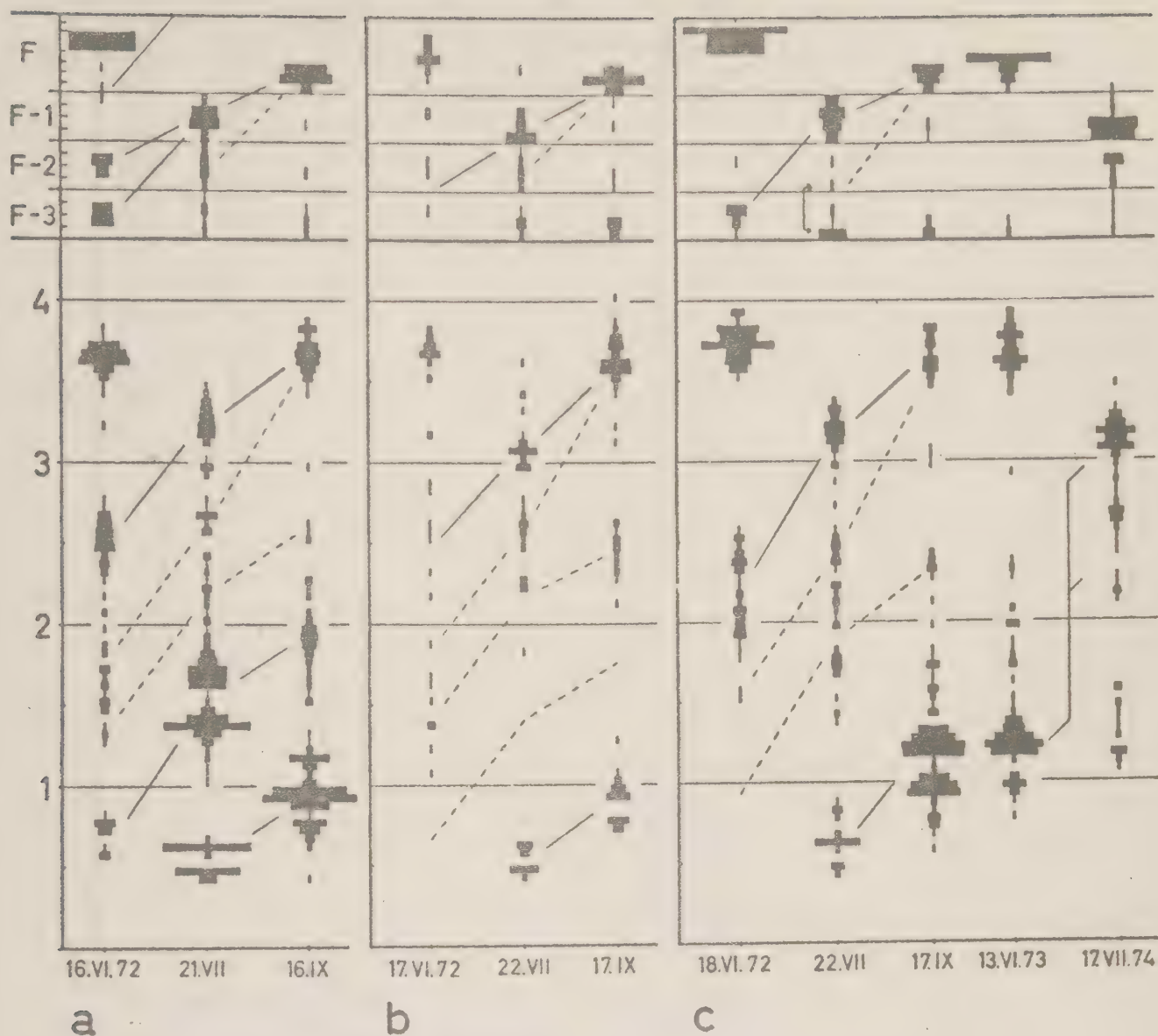


Fig. 2. Larval development of *C. hastulatum* at the northern study sites, $67^{\circ}50'N$. The graphs are designed as in Fig. 1. The different sampling dates are shown at the bottom. - (a) Site 1. - (b) Site 2. - (c) Site 3. - The main, reasonably certain development is indicated by solid lines, and the more uncertain interpretations are shown with broken lines. In (b), also the hypothetic development of the missing 1971 year-class is shown with a broken line. The instar assignment in the upper part is uncertain.



Fig. 3. Southern population. The effect of photoperiod and overwintering size on the time to entry into the F instar, and the duration of the latter, in an experiment started on 7 May 1974. The experimental temperature was 20°C. The symbols indicate the time of entry into the F instar at different photoperiods, the photophases of which are shown in the legend. The duration of the F stadium, if recorded, is shown by the vertical line above each symbol. The head width of each larva at the beginning of the experiment is found on the abscissa. The instar in which the experiment was begun, reflecting the number of ecdyses that was required to reach the F instar during the experiment, is shown for size-groups and for some individual larvae.

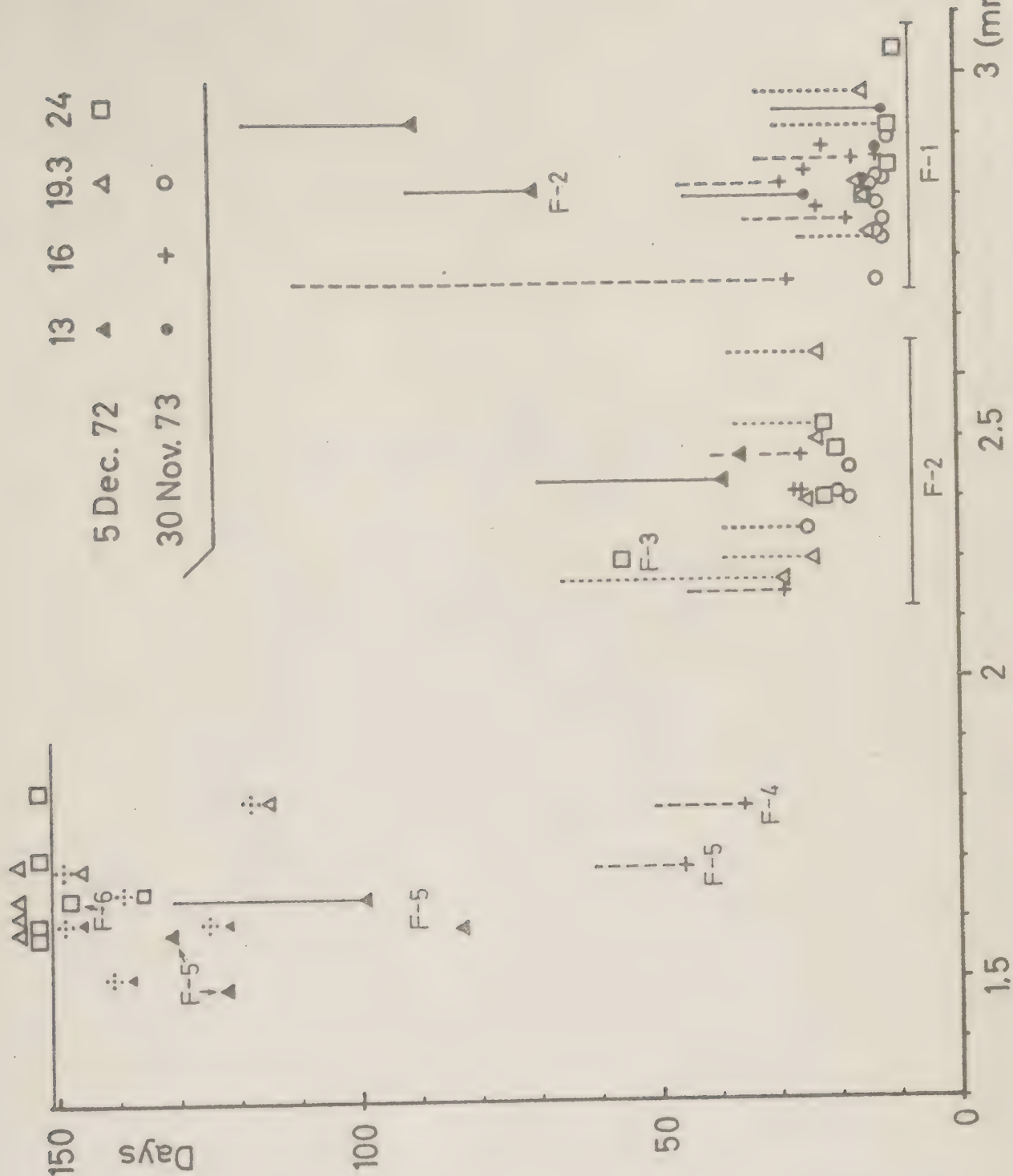


Fig. 4. Southern population. Same type of diagram as in Fig. 3, but based on different experiments (see legend). For reasons of space, the duration of the F stadium is shown only for part of the material. Larvae that did not reach the F instar during the 5 Dec. 1972 experiment are shown with symbols above a horizontal line at 151 days, which represents the end of the experiment. The death of larvae in instars earlier than F during the later part of that experiment are shown with small symbols and five dots. After 25 days, the 30 Nov. 1973 experiment was disturbed by a fault in the automatic switches, causing continuous light for one or two days.

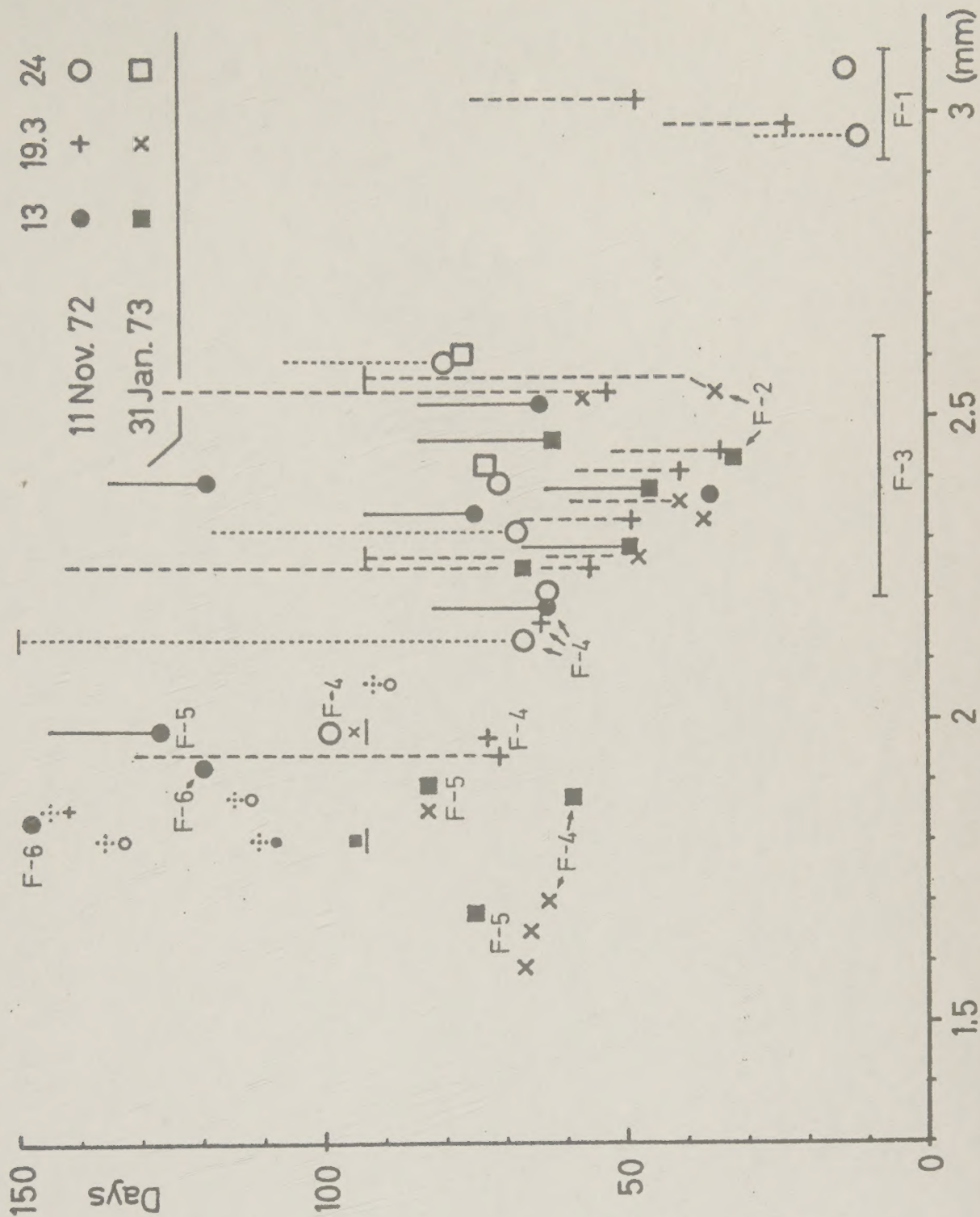


Fig. 5. Northern population. Same type of diagram as in Figs. 3 and 4. A short horizontal bar below a small symbol, or at the end of an F instar line, indicates the end of an experiment.

